REPORT ON BIOLOGICAL, MEDICAL AND BIOPHYSICS PROGRAMS

PART I - SEMIANNUAL REPORT OF RADIOLOGICAL PHYSICS DIVISION

PART II - QUARTERLY REPORT OF BIOLOGICAL AND MEDICAL RESEARCH DIVISION

January 1955

Please note that the work of the Radiological Physics Division has been included with that of the Biological and Medical Research Division in this report. The Radiological Physics Division is reporting for a six-month period, the previous report for this Division being included in ANL-5288, issued July 1954. Please note also that an index for the year appears at the end of this report. Preceding reports are:

ANL-5332 October 1954
ANL-5288 July 1954
ANL-5247 April 1954

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PART I

SEMIANNUAL REPORT OF RADIOLOGICAL PHYSICS DIVISION

FOR

JULY THROUGH DECEMBER 1954

John E. Rose
Director
PART I

TABLE OF CONTENTS

Dosimetry and Standardization

I. Ionization in heavy gases of low Z
   S. S. Brar and L. D. Marinelli ............... 5

II. Control apparatus for low- and high-level gamma rooms
    S. S. Brar and W. J. Eisler, Jr. .............. 6

Fast Neutron Dosimetry
   I. B. Berlman .................................. 7

Intake and Retention of Radium at Natural Levels
   A. F. Stehney and H. F. Lucas, Jr. ............. 8

Studies on Movement of Water in Soil
   H. F. Lucas, Jr. and A. F. Stehney .............. 16

Radium Toxicity

I. Measurement of radon partition by gamma-ray method
   P. F. Gustafson and L. D. Marinelli ............ 23

II. Use of scintillation counter for double tracer experiment
    P. F. Gustafson ................................ 26

III. The measurement in humans of Ra gamma-ray activities
     smaller than the activity of natural body potassium
     L. D. Marinelli, C. E. Miller, R. E. Rowland and
     J. E. Rose .................................. 27

Ionization in Pure Gases and the Average Energy to Make an Ion Pair for Alpha- and Beta-Particles
   W. P. Jesse .................................. 30

Drift Velocity of Electrons in Gases
   J. C. Bowe .................................. 40

Appendix I. Release of free electrons in a chamber: Experimental details
   J. C. Bowe .................................. 48
TABLE OF CONTENTS

Appendix II. A gastight chamber
J. C. Bowe and E. A. Mroz ................................. 50

Positive Ion Emission
H. A. Schultz .............................................. 53

Meteorology

Atmospheric diffusion studies ............................ 54
The electronic wind vane .................................. 58
Climatology .................................................. 58
Technical services .......................................... 61

Miscellaneous Activities ................................. 62
Publications .................................................. 63
DOSIMETRY AND STANDARDIZATION

I. Ionization in Heavy Gases of Low Z

S. S. Brar* and L. D. Marinelli

Two "air wall," guard-electrode type, cylindrical ionization chambers have been used to study the ionization in $C_4F_8$ gas and in air at equal pressures ($\sim 740$ mm Hg) when exposed to various X- and gamma-ray sources. The following tentative results are reported herewith:

<table>
<thead>
<tr>
<th>Source</th>
<th>kv</th>
<th>ma</th>
<th>Diaphragm</th>
<th>Filter</th>
<th>d</th>
<th>R</th>
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</thead>
<tbody>
<tr>
<td>X-rays</td>
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<td>6 mm</td>
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<td>8.9</td>
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<tr>
<td>X-rays</td>
<td>54</td>
<td>5</td>
<td>6 mm</td>
<td>None</td>
<td>200 cm</td>
<td>8.8</td>
</tr>
<tr>
<td>X-rays</td>
<td>86</td>
<td>5</td>
<td>6 mm</td>
<td>None</td>
<td>200 cm</td>
<td>8.6</td>
</tr>
<tr>
<td>X-rays</td>
<td>150</td>
<td>5</td>
<td>6 mm</td>
<td>None</td>
<td>200 cm</td>
<td>8</td>
</tr>
<tr>
<td>X-rays</td>
<td>200</td>
<td>5</td>
<td>6 mm</td>
<td>None</td>
<td>200 cm</td>
<td>8.5</td>
</tr>
<tr>
<td>X-rays</td>
<td>50</td>
<td>5</td>
<td>6 mm</td>
<td>Yes**</td>
<td>200 cm</td>
<td>8.4</td>
</tr>
<tr>
<td>X-rays</td>
<td>100</td>
<td>5</td>
<td>6 mm</td>
<td>Yes**</td>
<td>200 cm</td>
<td>7.6</td>
</tr>
<tr>
<td>X-rays</td>
<td>150</td>
<td>5</td>
<td>6 mm</td>
<td>Yes**</td>
<td>200 cm</td>
<td>7.4</td>
</tr>
<tr>
<td>X-rays</td>
<td>200</td>
<td>5</td>
<td>6 mm</td>
<td>Yes**</td>
<td>200 cm</td>
<td>7.3</td>
</tr>
<tr>
<td>Thulium</td>
<td></td>
<td>(average result of 4 different distances)</td>
<td>7.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesium$^{137}$</td>
<td></td>
<td>(average result of 2 different distances)</td>
<td>7.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt$^{60}$</td>
<td></td>
<td>(average result of 2 different distances)</td>
<td>7.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radium</td>
<td></td>
<td>(average result of 3 different distances)</td>
<td>7.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

where $R = \text{the ratio of } C_4F_8 \text{ chamber current to air chamber current.}$

We may provisionally conclude that a considerable gain in sensitivity can be achieved by using $C_4F_8$ instead of air without drastic departure from air equivalence. The ratios given above must be corrected for any difference

*Division of Biological and Medical Research, ANL.

**For filter sizes used see: Margarete Ehrlich and Stephen H. Fitch in Nucleonics 2 (3) 5 (1951).
in wall thickness; moreover, since difficulty was experienced in saturating the C₄F₈ gas chamber (above ratios were obtained by extrapolating the current to infinite collection voltage), further study is necessary. The currents were found to vary between one and two per cent according to the polarity used. The above data are for negative collection voltages.

II. Control Apparatus for Low- and High-Level Gamma Rooms

S. S. Brar* and W. J. Eisler, Jr.*

An independent background radiation monitoring system is being set up for low- and high level gamma rooms. The detector to be used for this purpose is a vibrating reed electrometer (60 cycle type, an old Argonne National Laboratory version) at its highest sensitivity with a resistor input. An integral part of this recorder is a circular Brown Recorder which also will be used to keep track of the time of exposure.

The problem has arisen as how to limit the current to the input of the electrometer when the ionization chamber is exposed to the bare Co⁶⁰ source. Thought has been given to shorting the input of the electrometer electrically by using the source gamma-rays as a trigger.

The use of cadmium sulfide crystal as a detector of gamma-rays is contemplated for the purpose. The output current of the crystal will be amplified sufficiently to operate a relay which will short the input electrometer resistor when the gamma source is in use. The current amplifier has been built for this purpose by the Electronics Group. The entire setup will be tried in the near future.

* Division of Biological and Medical Research, ANL.
FAST NEUTRON DOSIMETRY

Isadore B. Berlman

The monoergic D-D beam from the newly installed neutron generator was used to test two methods of obtaining a pure proton recoil, as previously discussed (Berlman, ANL-5288, pp. 114-117).

Briefly, the first method compared the response of two scintillating solutions, xylene and 1-4-bis (trifluoromethyl) benzene. With monoergic gamma-ray sources, the responses of the two solutions are matched so that by subtraction the gamma-ray background is eliminated. After the pulse heights of the solutions exposed to gamma-rays are matched, it is necessary to prove that the pulse heights of the two solutions are matched to proton recoils. This was verified when the 2.2-Mev neutrons from the neutron generator were used. It seems, therefore, that the method is valid for neutron energies up to 2.2 Mev. Tests with 14-Mev neutrons are being planned.

The second method for production of a pure recoil spectrum (i.e., free of gamma-ray background) from a neutron source consisted of comparing the two responses obtained in one scintillator when two attenuators, paraffin and graphite, were used. When proper volumes of the attenuators are chosen, the gamma-rays from the target are attenuated equally by the attenuators. The difference curve is then a pure recoil spectrum, similar to the one obtained by the first method, but of smaller magnitude.
INTAKE AND RETENTION OF RADIUM AT NATURAL LEVELS

A. F. Stehney and H. F. Lucas, Jr.

Summary of Data

The work at Stateville Penitentiary was concluded in July and all equipment was removed in September. Since last reported, measurements of radon in breath were made on 8 new subjects, 5 of whom had been at Stateville for a period of 4 to 7 years. In addition, 14 fecal samples from 6 subjects were analyzed for radium in order to obtain more data on the rate of intake. A summary of all data obtained at Stateville is given below.

The values of body radium for the subjects (Table I-1) were calculated from the rate of expiration of radon in the breath, assuming that 70 per cent of the radon from body radium is expired. These values include corrections for residual atmospheric radon in the breath which were based on the concentration of radon in room air, the length of time radon-free air was breathed, and the volume of air collected for each sample. Each value in the fourth column is the average of 2 or 3 determinations made during a single run of 16 hours or more; duplicate runs were made on 3 subjects. The estimates of the amount of radium retained from intake at Stateville only which are given in the last column, were compared with a power function expression for retention after a single dose, as described later.

Table I-2 gives the radium content of the fecal samples. As indicated previously,(1) it is believed that the rate of excretion of radium for these subjects is within a few per cent of the rate of intake. However, it was impractical to extend the sampling period beyond 3 days, because the subjects had to be housed in the prison hospital during this period. Thus, the average daily excretion could not be measured accurately, and it was necessary to consider the excretion time corresponding to each fecal sample as being equal to the length of time since the previous stool was passed. A mean value of $12.7 \times 10^{-12}$ g Ra per day was obtained, and the standard deviation of the mean was $2.4 \times 10^{-12}$ g Ra per day. For comparison, the mean for 3 non-Stateville subjects was $(1.7 \pm 0.4) \times 10^{-12}$ g Ra per day.

It is of interest to note that the average dry weight of the daily fecal excretion is consistent with values quoted in the literature.(2)

Power Function Expression for Retention of Radium

The data were analyzed in terms of the power function expression for retention of radium following a single dose which has been proposed by Norris, Speckman, and Gustafson.(3) Assuming a continuous intake rate, C,
TABLE I-1

Radium Content of Stateville Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Years On Ra Water</th>
<th>Units of $10^{-10}$g Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total Body Content*</td>
</tr>
<tr>
<td>HL</td>
<td>29</td>
<td>0.00</td>
<td>0.35 ± 0.07</td>
</tr>
<tr>
<td>RS</td>
<td>26.4</td>
<td>0.19</td>
<td>0.80 ± 0.09</td>
</tr>
<tr>
<td>RLM</td>
<td>24.6</td>
<td>0.20</td>
<td>0.96 ± 0.06</td>
</tr>
<tr>
<td>JFM</td>
<td>31.4</td>
<td>0.23</td>
<td>1.70 ± 0.24</td>
</tr>
<tr>
<td>AJG</td>
<td>24.6</td>
<td>0.24</td>
<td>0.72 ± 0.31</td>
</tr>
<tr>
<td>WF</td>
<td>25.6</td>
<td>0.24</td>
<td>0.77 ± 0.09</td>
</tr>
<tr>
<td>CSS</td>
<td>22.0</td>
<td>0.26</td>
<td>1.11 ± 0.10</td>
</tr>
<tr>
<td>JEF</td>
<td>22.6</td>
<td>0.32</td>
<td>0.88 ± 0.13</td>
</tr>
<tr>
<td>JMW</td>
<td>25.1</td>
<td>0.32</td>
<td>0.79 ± 0.11</td>
</tr>
<tr>
<td>BFB</td>
<td>26.0</td>
<td>0.28</td>
<td>4.34 ± 0.19</td>
</tr>
<tr>
<td>J LH</td>
<td>29.1</td>
<td>0.43</td>
<td>0.64 ± 0.29</td>
</tr>
<tr>
<td>CD</td>
<td>34.8</td>
<td>0.39</td>
<td>0.72 ± 0.15</td>
</tr>
<tr>
<td>ERJ</td>
<td>25.2</td>
<td>0.66</td>
<td>2.04 ± 0.13</td>
</tr>
<tr>
<td>FVG</td>
<td>47.6</td>
<td>3.8</td>
<td>2.79 ± 0.14</td>
</tr>
<tr>
<td>HA</td>
<td>26.3</td>
<td>4.2</td>
<td>1.00 ± 0.12</td>
</tr>
<tr>
<td>GB</td>
<td>33.1</td>
<td>4.4</td>
<td>2.17 ± 0.18</td>
</tr>
<tr>
<td>FS</td>
<td>31.1</td>
<td>6.7</td>
<td>3.42 ± 0.19</td>
</tr>
<tr>
<td>LJ M</td>
<td>37.3</td>
<td>7.3</td>
<td>1.21 ± 0.11</td>
</tr>
<tr>
<td>CG</td>
<td>45.8</td>
<td>10.0</td>
<td>2.00 ± 0.16</td>
</tr>
<tr>
<td>JB</td>
<td>52.2</td>
<td>10.0</td>
<td>1.71 ± 0.20</td>
</tr>
<tr>
<td>FAS</td>
<td>27.8</td>
<td>14.0</td>
<td>1.88 ± 0.15</td>
</tr>
<tr>
<td>LM</td>
<td>39.2</td>
<td>15.6</td>
<td>2.44 ± 0.51</td>
</tr>
<tr>
<td>RC</td>
<td>40.8</td>
<td>16.4</td>
<td>1.60 ± 0.13</td>
</tr>
<tr>
<td>LW</td>
<td>40.1</td>
<td>16.6</td>
<td>1.97 ± 0.12</td>
</tr>
<tr>
<td>RH</td>
<td>48.0</td>
<td>18.0</td>
<td>3.18 ± 0.16</td>
</tr>
<tr>
<td>RHC</td>
<td>35.7</td>
<td>19.0</td>
<td>2.60 ± 0.40</td>
</tr>
<tr>
<td>RCL</td>
<td>37.6</td>
<td>19.6</td>
<td>2.01 ± 0.37</td>
</tr>
<tr>
<td>JC</td>
<td>43.4</td>
<td>20.8</td>
<td>2.56 ± 0.23</td>
</tr>
<tr>
<td>CH</td>
<td>49.9</td>
<td>21.6</td>
<td>1.78 ± 0.09</td>
</tr>
<tr>
<td>CEI</td>
<td>46.6</td>
<td>22.1</td>
<td>1.28 ± 0.15</td>
</tr>
<tr>
<td>HH</td>
<td>48.6</td>
<td>22.6</td>
<td>1.64 ± 0.10</td>
</tr>
<tr>
<td>MJ</td>
<td>50.6</td>
<td>25.0</td>
<td>4.85 ± 0.28</td>
</tr>
</tbody>
</table>

* Errors shown are the average error of replicate measurements or the statistical 0.9 error in counting, whichever was the larger.
### TABLE I-2

Radium Content of Fecal Samples from Stateville Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Per Sample</th>
<th>Estimated Days</th>
<th>Per Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry Wt. (g)</td>
<td>$10^{-12}$g Ra</td>
<td>Per Sample</td>
</tr>
<tr>
<td>RS</td>
<td>17.9</td>
<td>12.1</td>
<td>1</td>
</tr>
<tr>
<td>JEF</td>
<td>23.7</td>
<td>7.3</td>
<td>1</td>
</tr>
<tr>
<td>JMW</td>
<td>65.5</td>
<td>21.2</td>
<td>1</td>
</tr>
<tr>
<td>BFB</td>
<td>46.8</td>
<td>23.1</td>
<td>2</td>
</tr>
<tr>
<td>FVG</td>
<td>8.6</td>
<td>13.6</td>
<td>2</td>
</tr>
<tr>
<td>LJM</td>
<td>3.4</td>
<td>3.9</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>9.5</td>
<td>8.4</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>52.6</td>
<td>39.9</td>
<td>1</td>
</tr>
<tr>
<td>FAS</td>
<td>6.1</td>
<td>4.1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8.4</td>
<td>3.6</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>10.3</td>
<td>1.9</td>
<td>0.5</td>
</tr>
<tr>
<td>LW</td>
<td>29.0</td>
<td>15.3</td>
<td>1.5</td>
</tr>
<tr>
<td>RHC</td>
<td>46.9</td>
<td>41.4</td>
<td>1</td>
</tr>
<tr>
<td>CEI</td>
<td>27.5</td>
<td>8.9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>19.2</td>
<td>7.0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46.6</td>
<td>12.9</td>
<td>1</td>
</tr>
<tr>
<td>MJ</td>
<td>14.8</td>
<td>16.5</td>
<td>2</td>
</tr>
<tr>
<td>Mean</td>
<td>26.6</td>
<td>14.2</td>
<td>-</td>
</tr>
<tr>
<td>$\sigma$ Mean</td>
<td>4.3</td>
<td>2.5</td>
<td>-</td>
</tr>
</tbody>
</table>
the increment of amount retained, dR, at time T after intake during an element of time, dT, may be expressed as though a single dose of amount CdT had been taken:

\[ dR = A C T^b dT \quad -1 < b < 0, \; T \geq 1 \]  

(1)

where A is the fraction retained at T equal to 1. Values of T less than 1 are not considered because retention shortly after intake cannot be handled with a simple mathematical expression, and, in particular, the expression has no meaning at \( T = 0 \). The value of b must be less than zero for retention after a single dose to decrease with time, and it must be greater than minus one for retention during continuous intake (cf. below) to be positive and increasing with time.

In order to evaluate the total retention, R, over a period from 0 to t, during which intake continues, it is necessary to integrate Equation (1). Since it cannot be integrated for the period of T less than one, an approximation may be made as follows:

\[ R = \int_0^t dR = \int_1^t dR + Q \]

(2)

where Q is the amount contributed from intake during the period of T less than 1. Thus,

\[ R = \frac{AC}{b + 1} (t^{b+1} - 1) + Q \]

(3)

The value of Q must lie somewhere between AC, which would be retained if a dose numerically equal to C were taken one unit of time before the time of measurement, and C, which would be the retention if that amount were taken just prior to the time of measurement. Note that using a value AC for Q would imply a redefinition of A as the fraction retained at the end of one unit of time during which radium was taken continuously. Accordingly, the true value of R may be expressed as:

\[ R > \frac{AC}{b + 1} (t^{b+1} + b) \]  

(4)

\[ R < \frac{AC}{b + 1} t^{b+1} + \frac{b+1-A}{A} \]  

(5)

If the value of \((b + 1)\) is not too small, i.e., if R increases appreciably with time, then for large values of t we have

\[ R \approx \frac{AC}{b + 1} t^{b+1} \]  

(6)
Thus, a plot of log $R$ vs log $t$ may provide a straight line portion at large values of $t$ which can be used to evaluate $(b + 1)$ as the slope and 

$$\frac{AC}{b + 1}$$

as the extrapolated intercept at unit time.

Since Equation (3) was derived for a continuous dose of constant intake rate, it was necessary to estimate the amount of radium retained only from intake at the prison by the subjects. For this purpose, it was assumed that the subjects contained the same amount of radium ($0.4 \times 10^{-10}$g Ra) as the control upon entering the prison, and that the rate of loss of that radium would be such as to leave one-half after 10 years.* The net values are given in Column 5 of Table I-1 and plotted in Figure I-1. Using the above interpretation of the slope and intercept the following values were obtained from the least squares line through the points:

$$b = -0.67, \text{ with } \sigma_b = 0.05$$

$$AC = 0.036 \times 10^{-10}\text{g days} \quad -(b + 1)$$

Taking the daily rate of intake, $C$, as equal to the mean value for excretion from Table I-2, we get

$$A = 0.28 \text{ in units of days}^{-b}$$

Using the above values for $b$ and $AC$, body radium values were calculated according to Equation (3) with $Q$ equal to $AC$. As plotted in Figure I-1, it is seen that even these minimum values are fairly close to the straight line plot for the uptake times used. The maximum values calculated from Inequality (5) were practically identical with the straight line plot for $t$ greater than 100 days.

Discussion of the Present Work

The limitations imposed by the low levels of radium encountered in this work and by difficulties associated with work conducted “in the field” at Stateville caused the present work to be incomplete in several important aspects at this time.

---

*For radium accumulated from continuous intake during time $t$, the fraction remaining $(T - t)$ units of time later is approximately

$$\frac{T^b + 1 - (T - t)^b + 1}{t^b + 1}.$$ For $t = 30$ yr and $T = 40$ yr, the fraction calculated using the value of $b$ (0.67) obtained in the present work is 0.4. This is only slightly lower than the fraction calculated from a preliminary estimate of the value of $b$, so a further approximation did not seem necessary.
Figure 1-1

Net Body Radium vs Time at Stateville Penitentiary

Least squares line corresponding to $R = \frac{AC}{b + 1} t^b + 1$

Plot of $\frac{AC}{b + 1} (t^b + 1 - 1) + AC$ calculated from values of $b = -0.67$, $AC = 0.108$ which were obtained from the least squares line.
Most obvious, of course, is the need for gamma-ray measurements or post-mortem analyses in order to determine the fraction of radon expired in the breath. If less than 70 per cent is expired, the true values of body content might be considerably higher than reported here. However, the fraction of radon expired from newly acquired radium has been reported as larger than that from older deposits, and, on the average, the radium burden of the Stateville subjects was acquired more recently than that of the Elgin subjects for whom the figure of 70 per cent was reported. Thus, a higher fraction might be expected in the present work, and an increase to even 100 per cent would not appreciably change the body radium values.

The fraction of atmospheric radon remaining in breath samples as a function of time of breathing radon-free air was calculated from the data of Harley, Jetter and Nelson. However, analyses of breath samples during the first 7 hours of the runs on the control subject in the present work indicated that atmospheric radon was being eliminated more slowly, presumably because of some rebreathing in the respiratory helmet and because the supplied air was not completely free of radon. If this be so, somewhat larger corrections for residual atmospheric radon should have been made, and the values of Column 4, Table I-1, would be too large by perhaps $0.2 \times 10^{-10}$ g Ra.

Finally, runs on additional subjects from Chicago should be made in order to obtain a more valid baseline for computing the retention of the Stateville subjects from radium ingested at the penitentiary.

**Comparison with Other Studies**

The range of body radium values found in the present work is practically identical to the range of $0.38 \times 10^{-10}$ to $3.53 \times 10^{-10}$ g Ra with an average value of $1.18 \times 10^{-10}$ g Ra found by Hursh and Gates at Rochester. This was not expected, because water at Rochester or New York contains as little radium as Chicago water. Although the Rochester subjects were considerably older than the Stateville subjects, a factor of two in age would result in only 1.3 times as much radium in the body according to the rate of increase predicted by the present work. Some data on the rate of excretion of radium by Rochester people would be useful in comparing the two studies.

Norris, Speckman and Gustafson found somewhat greater retention of radium in their work on subjects who had received intravenous injections. Their values for the constants in the power function expression would predict retention of about one-half one day after a single dose and an increase of body radium proportional to the square root of time for continuous intake, whereas the corresponding values in the present work predict about one-third retention and an increase proportional to the cube root of time. In view of the numerous differences in experimental conditions - Norris, et al., measured retention in 7 females and 1 male 20 years after several intravenous
doses totaling about 100 μg Ra, while we measured the increase in radium content of male subjects from oral ingestion of about $10^{-5}$ μg Ra per day - it is not obvious that the two sets of data should agree. Indeed, considering the scatter in both sets of data, it is not certain that they disagree.

Our thanks to Mr. Sylvanus A. Tyler of the Biological and Medical Research Division for the least squares analysis of the data and for discussions of the proper forms of the equations.

REFERENCES


STUDIES ON MOVEMENT OF WATER IN SOIL

H. F. Lucas, Jr. and A. F. Stehney

Introduction

This is a preliminary report on the rate of movement of water in soil as measured by using tritiated water as a tracer. The soil in the 300 Area where the tests were made is known as till, a glacial drift consisting of an unassorted mixture of clay, sand, gravel and boulders. Near the surface, the soil is composed of yellowish oxidized clay extending to a depth of about 12 feet and blue-grey unoxidized clay below this.

The tracer was introduced at a depth of 13 feet below the surface and two years later no tritiated water was found more than 6 feet from the injection site within the detection limits of 0.02 per cent of the original tracer concentration. It seems evident then, that fission products would travel very slowly in this soil if we follow the reasonable assumption that solutes will not travel faster through soil than the water in which they are dissolved.

Experimental Procedures

In order to maintain natural conditions, field experiments were performed in preference to laboratory studies on soil samples. Nine test holes (wells) were drilled to depths varying from 12 to 18.5 feet in the arrangement shown in Figure I-2. Each test hole was constructed with a 2-inch galvanized iron pipe casing, and a 1.5-foot long perforated metal screen at the bottom. The screen permitted water to move freely between the well and the surrounding soil. After construction, the test holes were allowed to stand until the water levels in the casings reached an equilibrium height.

The analysis for tritiated water was made by allowing the vapor from 0.10 ml of water to pass over zinc heated to approximately 400°C. The hydrogen thus formed was transferred to a cylindrical counter of about 700 cc volume which was operated in the Geiger region.

Movement of Tritium to Outer Wells

During the first phase of the experiment, 1.07 millicuries of tritiated water in 5 liters of tap water were introduced at the bottom of the central well (No. 1) through rubber tubing. Under the head provided by the extra water, enough water left the well in 10 days to distribute itself to a distance of about 1 foot in the soil surrounding the well bottom. Subsequently, samples of water were taken at intervals from the other wells and analyzed for tritium.
Figure I-2

Plot of the Test Holes in the 300 Area
content. The times required for tritiated water to travel from the injection site to the other wells are given in Table I-3. Tritium was first detected in Well 1A (the closest well) 250 days after the start of the experiment. Sampling of the observation wells was continued until at 660 days tritium was detectable in all four of the two-foot radius wells. None was found in any of the four-foot radius wells.

TABLE I-3

Tritium in Water Samples from Test Holes

<table>
<thead>
<tr>
<th>Elapsed Time Days</th>
<th>Counts/Minute/0.10 ml Water in Test Holes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1A</td>
</tr>
<tr>
<td>0 - 235</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>250</td>
<td>2.5 ± .8</td>
</tr>
<tr>
<td>272</td>
<td>2.6 ± .5</td>
</tr>
<tr>
<td>292</td>
<td>4.8 ± .8</td>
</tr>
<tr>
<td>323</td>
<td>8.6 ± .6</td>
</tr>
<tr>
<td>641</td>
<td>43. ± 1.0</td>
</tr>
<tr>
<td>662</td>
<td>40. ± 1.1</td>
</tr>
<tr>
<td>689</td>
<td>36. ± 1.0</td>
</tr>
</tbody>
</table>

No tritium above 2.0 CPM was detected in holes #5A, 6A, 7A, and 8A at any time.

* Sample lost.

3 CPM = 0.02 per cent initial tracer concentration.

Distribution of Tritium in Soil

The second phase of the experiment, determining the distribution of tritiated water in the soil below the test wells, was started about 700 days after injection of the tritiated water. Samples of soil were obtained by driving a coring tube into the soil below the test hole after the water had been pumped from the wells. The coring tube was a 1-1/4-inch diameter steel pipe, 2-1/2 feet long, which was coupled to a pipe of sufficient length to permit driving from the surface. A movable plug placed in the tube helped prevent the entrance of water and loose dirt while the tube was being inserted into the test well; this plug was especially needed when successive corings were made through the hole left by the previous core sample. Unfortunately,
the soil samples obtained were usually only 1/2 to 3/4 as long as the length of the drive (Table I-4), because the friction of the plug and soil within the tube prevented free entry of soil. Thus, it was impossible to determine the true depth of a core sample from its position in the coring tube. However, the maximum depth of the sample could be obtained by assuming that all of the soil sample obtained entered the core tube during the last portion of the drive. This assumption resulted in assigning upper limits to the distance of travel of tritiated water.

The core tubes were cut into sections 2 inches long with a pipe cutter. The soil was left in the sections which were stored in sealed containers until it was convenient to distill the water from the soil for the tritium measurement.

No tritium was found in any of the soil cores from the four wells located 4 feet from the injection well. For each of the other wells, measurable activity was found throughout the first core (Figure I-3) and part of the second core (Figure I-4). The maximum distance of penetration of tritiated water under each well was taken from Figure I-4 as the point at which no activity was found. However, the assumption that all the soil obtained in each core entered in the last portion of the drive resulted in a discontinuity between the depths, assigned to the bottom of the first core and the tops of the second core, and some soil probably entered the coring tube as it was inserted through the hole left by the first core. Thus, the depths of penetration taken from Figure I-4 are too large by some unknown amount. An alternate set of values was obtained from the plot of activity vs depth for the first core by extrapolation to zero activity as shown in Figure I-3. This procedure eliminated contamination errors but did not provide as certain an upper limit for distance of travel as the direct observation of zero activity in the second core. Radial distances from the bottom of the injection well to the depth at which no activity was found under each well are given in Table I-5 for both procedures.

Conclusion

The rate of movement of water through the soil as observed in this experiment may be interpreted as resulting either from flow under a gradient or as self-diffusion of water. Therefore, extrapolation to greater depths will depend on the model chosen, as well as on soil water studies of this soil which have been made. For the simple case of linear extrapolation, it would require 30 years for water to travel from the surface to the Niagara limestone which is located 100 feet below the surface and is the major aquifer for shallow wells in the vicinity of the laboratory.
<table>
<thead>
<tr>
<th>Test Hole #</th>
<th>Sea Level Elevation of Bottom (Ft)</th>
<th>Core #</th>
<th>Length Drive (Ft)</th>
<th>Length Core (Ft)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Injection)</td>
<td>731.6</td>
<td>1</td>
<td>2.5</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3.0</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3.0</td>
<td>1.9</td>
</tr>
<tr>
<td>1A</td>
<td>732.2</td>
<td>1</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3.6</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3.1</td>
<td>1.5</td>
</tr>
<tr>
<td>2A</td>
<td>732.2</td>
<td>1</td>
<td>2.5</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3.0</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3.6</td>
<td>0.7</td>
</tr>
<tr>
<td>3A</td>
<td>732.7</td>
<td>1</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
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<td>1.7</td>
</tr>
<tr>
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<td>732.8</td>
<td>1</td>
<td>2.2</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2.2</td>
<td>1.7</td>
</tr>
<tr>
<td>5A</td>
<td>726.2</td>
<td>1</td>
<td>2.5</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2.5</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3.5</td>
<td>1.9</td>
</tr>
<tr>
<td>6A</td>
<td>729.3</td>
<td>1</td>
<td>2.3</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2.7</td>
<td>1.8</td>
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<td></td>
<td></td>
<td>3</td>
<td>2.8</td>
<td>1.3</td>
</tr>
<tr>
<td>7A</td>
<td>729.4</td>
<td>1</td>
<td>2.4</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2.1</td>
<td>1.9</td>
</tr>
<tr>
<td>8A</td>
<td>730.8</td>
<td>1</td>
<td>2.4</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2.1</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3.0</td>
<td>1.6</td>
</tr>
</tbody>
</table>
Figure 1-4

Tritium Activity of the Water in the Samples from the First Core
- o- Measured values
- -- Extrapolation to zero activity

Figure 1-3

Tritium Activity of the Water in the Samples from the Second Core
- o- Measured values
### TABLE I-5*

Limit of Tritium Transport after 700 Days

<table>
<thead>
<tr>
<th>Test Hole #</th>
<th>Zero** Figure II</th>
<th>Radial Distance (Ft)</th>
<th>Zero** Figure III</th>
<th>Radial Distance (Ft)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Injection)</td>
<td>-†</td>
<td>-</td>
<td>726.3</td>
<td>5.3</td>
</tr>
<tr>
<td>1A</td>
<td>728.7</td>
<td>3.1</td>
<td>726.8</td>
<td>4.9</td>
</tr>
<tr>
<td>2A</td>
<td>729.3</td>
<td>3.1</td>
<td>727.2</td>
<td>4.7</td>
</tr>
<tr>
<td>3A</td>
<td>727.7</td>
<td>2.9</td>
<td>727.8</td>
<td>4.4</td>
</tr>
<tr>
<td>4A</td>
<td>729.8</td>
<td>2.8</td>
<td>729.0</td>
<td>3.3</td>
</tr>
</tbody>
</table>

* No tritium was detected in the soil samples from below Wells 5A, 6A, 7A, and 8A.

** Units of height above sea level in feet.

† Insufficient number of points to permit extrapolation.
RADIUM TOXICITY

I. Measurement of Radon Partition by Gamma-Ray Method

P. F. Gustafson* and L. D. Marinelli

Consideration has been given to the use of the gamma-ray emitted during the decay of Ra$^{226}$ as a means of measuring the fractions of radon retained and exhaled in mammals without the need of taking breath samples. This gamma-ray occurs in about only 6 per cent of the disintegrations and is internally converted about half the time, resulting in the emission of the K$\alpha$-ray of Rn$^{222}$. The energy of the primary gamma is 186 kev and that of the characteristic x-ray is 85 kev.

In spite of the low intensity of this gamma-ray, the method has been tried and has proved satisfactory. Two effects had to be studied simultaneously before actual partition values could be obtained for living animals. These are the relative intensity of the 186-kev photoline, both as a function of the RaB gamma-radiation present and as a function of the amount of absorbing and scattering material present. The first was determined by de-emanating a Ra$^{226}$ chloride solution and observing the intensity ratios as a function of time. The second was studied by placing the radium solution at different depths in a "pseudwood" phantom of suitable dimensions, always keeping the source to counter distance the same. Intensity values were obtained by the use of a scaler in conjunction with a single channel pulse height analyzer. The detector was 1/2 in. x 1-1/2 in. NaI(Tl) crystal and a DuMont 6292 photomultiplier.

Instead of taking a complete spectrum at each point in time and with each thickness of "pseudwood" used, only three regions of the spectrum were observed. These regions or channels are shown in Figure I-5 which shows the entire spectrum from 25 to 400 kev of a radium source containing about 5 per cent radon. Channel A contains the valley between the photoline and the K$\alpha$-ray as well as the entire photoline itself, Channel B just the photoline, and Channel C the three photolines from radium B at 241, 294, and 351 kev.

De-emanation was done by placing a vial containing the radium solution in a water bath and boiling for several hours. This served to drive off the radon as it was formed and it allowed also the radon daughters to decay out to a large extent. The vial was then sealed, the spectrum was taken and a count made to determine the total amount of activity present. In the latter, counts in the C channel were compared with those from a known radium standard; this allowed the computation of the completeness of de-emanation to be made.

*From the Division of Biological and Medical Research, ANL.
This procedure was followed before and after each run so that correction for growth of radon during the run might be made.

Since retention of radon by a living system does not seem to exceed 50 per cent, and since the sensitivity of this method decreases rapidly above 40 per cent retention, readings were not carried out for more than 4 days after the sealing of any one sample. The data taken in Channels A and B were each normalized by dividing by the value in Channel C. The product of these two normalized quantities was then taken. Figure 1-6 shows a plot of the magnitude of this product \((A \times B/C^2)\) as a function of per cent of radon equilibrium for 0, 2.8, 4.8, and 7.5 centimeters of "presdwood." Other ways of plotting this relationship may prove more advantageous but have not been tried as yet.

As an actual check of the method using experimental animals, three rabbits and a dog were measured prior to sacrifice. The dog was injected with radium almost 3 years ago and gave a value of radon retention between 25 and 29 per cent, in good agreement with the value of 28 per cent gotten by taking breath samples and total body gamma-ray readings on the same dog. A high radium dose level, young rabbit gave a value of 7 per cent 3 days after injection and a value between 13 and 14 per cent 17 days after injection at which time it was sacrificed. Two adult rabbits, one high level and one low level, 17 days after injection gave values of 18 to 19 and 21 to 23 per cent, respectively, in agreement with results obtained by Norris and Speckman on rats. All these animals have been sacrificed and are being followed by scintillation counting to determine per cent of radon equilibrium at time of sacrifice. The spread in radon values, most pronounced in the case of the dog, are due to the slow variation of the product function with radon retention at higher retention levels as well as to the slight dependence of the function on depth in the phantom. More animals will be run and checks will be made by either taking radon breath samples along with total body gamma-ray measurements, or by obtaining the equilibrium values with animals that have been sacrificed. The radon partition at time of sacrifice can then be gotten by subtracting the gamma measurement at time of sacrifice from the equilibrium gamma value.

The dependence of the product function on depth in the phantom is surprisingly small and lends encouragement for use of this method with humans. However, further work with a larger phantom must be carried out before this can be considered.

We are indebted to Dr. W. P. Norris and Mr. T. W. Speckman of the Biological and Medical Research Division for their cooperation in evaluating the pertinent parameters in the living animals.
II. Use of Scintillation Counter for Double Tracer Experiment

P. F. Gustafson*

A method counting simultaneously $^{32}$P and $^{51}$Cr has been tried and has proved successful. A 3-inch well type NaI(Tl) crystal was used as the detector in conjunction with a single channel pulse height analyzer. $^{32}$P is a pure beta emitter whereas $^{51}$Cr decays by electron capture with the subsequent emission of a gamma-ray, no betas occurring at all. The radioisotopes were in solution and were placed in plastic test tubes for counting in the well crystal. The well has a brass liner of sufficient thickness to stop all the betas from $^{32}$P, but offers little absorption for the $^{51}$Cr gamma-ray. Therefore all counts from $^{32}$P must be due to bremsstrahlung either in the solution or in the test tube and brass lining.

By counting on the photopeak of the 0.330 Mev gamma it is possible to count $^{51}$Cr with only slight interference from $^{32}$P. However, to count $^{32}$P efficiently it is necessary to count in a region of the spectrum where the Compton distribution from $^{51}$Cr is quite intense. In order to determine the number of counts due to each isotope in a mixture of the two, the following procedure was followed. A solution of $^{32}$P was counted with all pulses above 50 kev being recorded. The number of counts from $^{32}$P in the region 100 to 200 kev and 300 to 350 kev was determined. It was then possible to calculate the fraction of the total number of counts from $^{32}$P occurring in the two channels. A similar procedure was followed for $^{51}$Cr. Then a mixture of the two isotopes was counted, using only the two channels. The amounts of $^{32}$P and $^{51}$Cr were determined by solving two simultaneous equations. One for the fraction of each isotope in the first channel, the other for the fraction of each in the second channel. The results obtained for each isotope agreed within 5 per cent of the known value of each activity placed in the mixture.

Although the method is not new, it has been extended to measure a beta emitter by its bremsstrahlung. Further work regarding the effect of solids in the solution on production of bremsstrahlung will be carried out. Use of the method involving three isotopes is being contemplated. The third isotope will be $^{131}$I.

I wish to thank W. P. Norris and T. W. Speckman for the use of their well type NaI(Tl) crystal.

* From the Division of Biological and Medical Research, ANL.
III. The Measurement in Humans of Ra Gamma-Ray Activities Smaller than the Activity of Natural Body Potassium

L. D. Marinelli, C. E. Miller, R. E. Rowland and J. E. Rose

Considerable amount of effort has been dedicated to the quest of a method capable of measuring the gamma-ray activity of about $10^{-10}$g of Ra contained in the average human body. It is not feasible to present fully our findings to date. A brief presentation was given at the meeting of the Radiological Society of North America meeting in Los Angeles in December. The abstract, to be published in the January 1955 number of Radiology, follows:

***

ABSTRACT

The Measurement in Vivo of Ra Gamma-Ray Activities Lower than K$^{40}$ Levels Existing in the Human Body

L. D. Marinelli, C. E. Miller, R. E. Rowland and J. E. Rose

The measurement of gamma-rays emitted by more than about 0.03 $\mu$g of Ra in the human skeleton can be carried out conveniently with moderate shielding ($\sim$1/4 in. Pb) and easily available NaI crystals (2-1/4 in. x 1-1/2 in.) by comparing the counting rates engendered by suitably located persons with positive and negative radium exposure history. The error due to the difference in body potassium content ($\sim$0.012 gamma-ray $\mu$c) is small, if care is taken to match body weights. Below this level of burden, the task is made progressively more difficult by the stricter requirements of high intrinsic sensitivity, low environmental gamma-ray background and interference from K activity and cosmic rays. In our laboratory, crystals of NaI (1.5 in. x 1 in., 4.2 in. x 1.5 in.), CsI(1.5 in. x 1 in.) and a large plastic scintillator (6 in. x 5 in.) have been housed in a 7 ft x 7 ft x 8 ft room shielded by 8 in. Fe.

Their scintillation pulses have been analyzed in the presence of background, Ra and K sources under a variety of conditions in order to explore soundness and economics of detector design.

Ra body content can be determined by detection of 1.5- to 2.5-Mev pulses - to exclude all interference from K$^{40}$ - with a standard deviation of $\pm 1.4 \times 10^{-3}$ $\mu$c when the large NaI
crystal is used for an observational period of 2 hours. Results obtained with the small NaI and CsI crystals suggest that this limit cannot be reduced substantially by use of the latter and that approach along these lines is apt to be prohibitively expensive. A more promising method – capable of yielding in a 2-hour reading a standard deviation of \( \pm 3.4 \times 10^{-4} \mu\text{c} \) in the measurement of radium and 4.8 g in the measurement of K – is obtained by the simultaneous recording of pulses generated in the plastic and large NaI scintillators, provided they are suitably selected in each for maximum relative sensitivity to K and Ra, respectively.

Although preliminary experiments suggest that modest additional Hg shielding and cosmic ray coincidence techniques can substantially reduce the background counting rate, it seems at this time that the intrinsic radioactivity of the detector itself represents the major obstacle to the attainment of fluctuations of the order of one-tenth of those hitherto considered. In order to attain practical sensitivities the detector and its housing must be bulky – to intercept a significant fraction of the few gammas emitted by the human body – and must, at the same time, possess a total radioactivity lower than \( 10^{-12} \text{C} \).

***

We may add that in Figure 1-7 and Figure 1-8 are summarized the data taken respectively with the two large NaI and plastic scintillators. The K and Ra sources at 40 cm represent closely the counting rates these sources would yield if they were distributed in the adult human body placed under standardized conditions in the vicinity of the detectors. It is worthy of note that preliminary findings on a few humans show directly that the bulk of the gamma-radiation from the body originates from K\(^{40}\). As yet approximate calculations are consistent with the latest data gathered on the subject by Burch and Spiers,\(^{(1)}\) Sievert,\(^{(2)}\) and Reines, et al.\(^{(3)}\) The counting rates in the last channel (extreme right) denote pulses of energy higher than those available from Ra.

REFERENCES


Figure I-7 Pulse Height Analysis in 8" Fe Room
NaI - 4" x 1.5"

Figure I-8 Pulse Height Analysis in 6" Fe Room
Plastic - 6" x 4"
IONIZATION IN PURE GASES AND THE AVERAGE ENERGY TO MAKE AN ION PAIR FOR ALPHA- AND BETA-PARTICLES

William P. Jesse

Introduction

The long range research program to determine the mean energy to make an ion pair as a function of the gas used and of the kind of ionizing particle has been continued. Good progress has been made along two lines of approach. The first is a comparison of the relative ionization produced in very pure gases by alpha- and by beta-particles and the relative mean energy to form an ion pair for these two particles. Since a paper covering this phase of the work has just been sent for publication to the Physical Review, such work is presented here only in the form of an abstract.

The second phase of the work has consisted in the measurement of the average energy to make an ion pair for alpha-particles in mixtures of gases. Some very striking effects have been obtained, where minute additions of other gases to the noble gases increase greatly the ionization observed. Preliminary reports of this work have already been published.\(^1\),\(^2\)

Abstract

A series of measurements has been made of the relative currents produced in different gases by beta-particles from \(\text{Ni}^{63}\) and from tritium sources in an ionization chamber. In all cases only relative current measurements with argon as a standard gas have as yet been made. The value of \(W\), the average energy to make an ion pair computed relative to argon as a standard, is found to be the same for \(\text{Ni}^{63}\) and tritium sources. If these relative \(W_\beta\) values are plotted as abscissae against previously determined \(W_\alpha\) values for polonium alpha-particles as ordinates, a marked difference is observed in the gases investigated. For hydrogen and the noble gases the plotted points lie closely on a 45-degree straight line through the origin. Thus for these gases the ratio \(W_\alpha/W_\beta\) is constant. This constant may well be unity, but this is not proved as yet by these results. For all other gases so far investigated, the plotted points lie above the 45-degree line, indicating a higher efficiency of ionization (and a lower \(W\)) for the beta-particles than for the polonium alpha-particles. These results are in accord with the findings of Gray\(^3\) and Gurney.\(^4\) Gurney’s results have been extended here to include a greater variety of gases for reduced alpha-particles of approximately 1-Mev energy. Two postulates are advanced to explain the behavior of \(W_\alpha\) and \(W_\beta\) here found.
Alpha-Particle Ionization in Mixtures of Gases

In the references cited\(^{(1,2)}\) a brief account has already been given of the apparatus and method employed in measuring the ionization produced by single polonium alpha-particles in mixtures of gases. In general, one adds to a noble gas contaminating gases in concentrations of the order of a few parts in 10,000. The ionization increases at first very rapidly with the addition of contaminant and then more slowly until at last a saturation condition is reached, where the further addition of contaminant produces little or no further increase in ionization.

A plausible explanation for the increase in ionization observed is that this increase results from the transfer of energy from the metastable states in the noble gas when an excited atom collides with an atom of the contaminant. In the process the contaminant atom is ionized and a pair of ions collected in the chamber. Thus for argon as a contaminant in helium we have

\[
\text{He}^* + A \rightarrow \text{He} + A^+ + e^-.
\]

Any additional energy imparted by the metastable helium atom \(\text{He}^*\) to the argon atom \(A\) above the energy necessary to produce an ion pair is carried off as kinetic energy in the ejected electron \(e^-\).

It is not possible by the present method to measure the absolute molecular cross section for this ionizing encounter between the metastable atom and the impurity atom. It is, however, possible to measure quite accurately the ratio \(\frac{Ki}{Kd}\), where \(Ki\) is the cross section for the ionizing encounter above, and \(Kd\) is the cross section for deactivation of the metastable atom in the absence of any impurity. Thus in pure helium the metastable states must be discharged by some mechanism which does not result in the production of ion pairs. The exact mechanism by which this is accomplished is not as yet clear.

In the first of the references cited a simple method is given for computing the ratio \(\frac{Ki}{Kd}\) from the data of this experiment. The latest values so far obtained for \(\frac{Ki}{Kd}\) are given in vertical Column 3 of Table I-6 from directly measured values in Column 2. The pressure used in the helium mixtures was about 87 cm of mercury and for the neon values about 30 cm.

Since this experiment only the ratio \(\frac{Ki}{Kd}\) can be measured, no estimate of the absolute values of the cross sections can be made solely from the data here obtained. Biondi\(^{(5)}\) however, has made a measurement of \(Kd\) involving the metastable atoms in pure helium and neon found in a low-voltage
### TABLE I-6

Evaluation of Collision Cross Sections for Metastable Atoms

<table>
<thead>
<tr>
<th>Gas Mixture</th>
<th>Concentration of Contaminant for Half Metastables Converted</th>
<th>$\frac{K_i}{K_d}$ = Reciprocal Value in Previous Column</th>
<th>Absolute Collision Cross Sections - Cm$^2$ (He$^*$ on He = $9.6 \times 10^{-21}$ cm$^2$)</th>
<th>Biondi Cross Sections - cm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$ in He</td>
<td>$3.4 \times 10^{-4}$</td>
<td>$2.9 \times 10^3$</td>
<td>$1.5 \times 10^{-17}$ cm$^2$</td>
<td>$9.7 \times 10^{-17}$</td>
</tr>
<tr>
<td>A in He</td>
<td>$1.54 \times 10^{-4}$</td>
<td>$6.5 \times 10^3$</td>
<td>$8.4 \times 10^{-17}$ cm$^2$</td>
<td></td>
</tr>
<tr>
<td>Kr in He</td>
<td>$0.66 \times 10^{-4}$</td>
<td>$15.2 \times 10^3$</td>
<td>$20.0 \times 10^{-17}$ cm$^2$</td>
<td></td>
</tr>
<tr>
<td>Xe in He</td>
<td>$0.48 \times 10^{-4}$</td>
<td>$20.8 \times 10^3$</td>
<td>$28.0 \times 10^{-17}$ cm$^2$</td>
<td></td>
</tr>
<tr>
<td>CO$_2$ in He</td>
<td>$0.83 \times 10^{-4}$</td>
<td>$12.0 \times 10^3$</td>
<td>$16.0 \times 10^{-17}$ cm$^2$</td>
<td></td>
</tr>
<tr>
<td>Hg in He</td>
<td>$0.015 \times 10^{-4}$</td>
<td>$670 \times 10^3$</td>
<td>$900 \times 10^{-17}$ cm$^2$</td>
<td></td>
</tr>
<tr>
<td>H$_2$ in Ne</td>
<td>$4.7 \times 10^{-4}$</td>
<td>$2.1 \times 10^3$</td>
<td>$8.1 \times 10^{-17}$</td>
<td>$26 \times 10^{-17}$</td>
</tr>
<tr>
<td>A in Ne</td>
<td>$2.3 \times 10^{-4}$</td>
<td>$4.3 \times 10^3$</td>
<td>$45 \times 10^{-17}$</td>
<td></td>
</tr>
<tr>
<td>Xe in Ne</td>
<td>$0.43 \times 10^{-4}$</td>
<td>$23 \times 10^3$</td>
<td>$270 \times 10^{-17}$</td>
<td></td>
</tr>
</tbody>
</table>
arc. It is not at all certain that the mechanism of deactivation of the metastable states is the same in the experiment of Biondi as in the present one. However, an assumption that the absolute cross sections for $K_d$ for He and Ne of $9.6 \times 10^{-21}$ cm$^2$ and $8.9 \times 10^{-20}$ cm$^2$, respectively, apply to the present case yields the absolute values for $K_i$ given in vertical Column 4 of Table I-6. The agreement is fairly good with the three values of $K_i$ (Column 5) determined directly by Biondi. In spite of this apparent agreement it would seem that the values in Column 4 should be treated with some degree of reserve except for order of magnitude.

It is interesting to note that, with the exception of mercury, the molecular cross sections of the order $10^{-16}$ to $10^{-17}$ cm$^2$ are about those obtained for molecular cross sections from the data of kinetic theory. The value for mercury as an impurity, however, is of the order of $10^{-14}$ cm$^2$, about one hundred times as large as the other values. At present no reason can be given for this extremely large cross section.

**Variation of $\frac{K_d}{K_i}$ with Pressure of Gas Mixture**

It has been suggested by Burhop(6) that the mechanism of deactivation of the metastable atoms of helium in pure helium follows the reaction

$$\text{He}^* + \text{He} + \text{He} \rightarrow \text{He}_2^{\text{excited}} + \text{He}$$

$$\rightarrow \text{He}_2^{\text{ground}} + \text{He} + h\nu$$

$$\rightarrow \text{He} + \text{He} + \text{He} + h\nu$$

Here a metastable atom reacts in the presence of two neutral helium atoms to form molecular helium in an excited state. The excited state radiates a quantum of energy and goes to the ground state with immediate molecular dissociation.

In the three-body reaction above the probability of the reaction is a compound probability and hence varies as the square of the pressure. Thus,

$$\text{Probability of reaction} = K p^2 = (K_p) p.$$  

For convenience we may consider $(K_p)$ as a cross section for interaction, which is a variable with pressure. We may thus identify $(K_p)$ with the $K_d$ used in Reference 1. If the deactivation follows the reaction above,

*In Reference 1 a distinction was made between $\frac{K_d}{K_i}$ and $\frac{K_2}{K_1}$ which refer to the same ratio derived by different methods. This distinction is now dropped and for simplicity the notation $\frac{K_d}{K_i}$ will be used throughout.*
however, $K_d$ should change in direct proportion to the pressure and the ratio $\frac{K_d}{K_i}$ in the same proportion. The reciprocal $\frac{K_i}{K_d}$, which is more commonly used in Reference 1, then decreases with pressure.

Experiments have been carried out to determine whether the dependence on pressure predicted by such a method of deactivation of metastable states really exists in helium mixtures. In order that measurements over a relatively large range of pressures might be made without unduly large pressures in the apparatus, the energy of the alpha-particle used was markedly decreased. The experimental arrangement is shown in Figure I-9. A collimated beam of alpha-particles from a thin layer of Am$^{241}$ on a platinum plate passed through a mica window into the ionization chamber. The thickness of the mica was so chosen that the initial energy of the partially stopped alpha-particles was of the order of 1 Mev. For each mixture of gases used, the current through the ionization chamber was determined, and the ratio of this current to the corresponding current for very pure helium computed.

![Figure I-9 Source and Ionization Chamber](image-url)
In Figure I-10 are shown two curves taken at different pressures for helium-nitrogen mixtures. Here one plots as abscissae the concentration of nitrogen in helium in parts per 10,000 by volume. As ordinates are plotted the ratio of the ionization current obtained for any given mixture to the ionization obtained in pure helium. The latter value is rated unity on the ordinate scale. It will be seen that the curve for a pressure of 48 cm of mercury is higher than that for 110 cm of mercury at the lower concentrations of N₂, but the two curves tend to merge as the concentration becomes relatively large. Similar curves have been obtained for argon in helium and for hydrogen in helium.

By the use of the Stern-Volmer plot(1) one can determine the variation of $\frac{K_d}{K_i}$ as a function of pressure from the type of measurement shown in Figure I-10. In Figure I-11 the variation with pressure of $\frac{K_d}{K_i}$ is shown for mixtures of helium with argon, nitrogen, and hydrogen. The plots are seen to be straight lines with intercepts on the $\frac{K_d}{K_i}$ axis. If the three-body reaction, leading to excited He₂, were the only process for deactivation of the He* metastable states, we should expect a relation $\frac{K_d}{K_i} = \text{constant} \times p$, the equation of a straight line through the origin. Such a relation has already been found in He by Phelps and Molnar.(7) In the present case we have a relation $\frac{K_d}{K_i} = cp + c'$ where c and c' are constants. Thus as the pressure decreases to very low values $\frac{K_d}{K_i}$ approaches a constant value c', indicating another process of deactivation independent of pressure. From each of the graphs it may be noted that at a pressure of about 87 cm of mercury cp is about equal to c'. This would indicate that at this pressure the cross section ratio $\frac{K_d}{K_i}$ is made up of about equal parts of pressure dependent and pressure independent deactivation processes.

It is unfortunate that the present experiment can give no clue as to the nature of the pressure independent process. It may well be that under impacts with another atom of He normally forbidden transitions can take place from the atomic metastable states, and thus deactivation can occur. That this is the nature of the process, while reasonable, is neither proved nor disproved by these results.

For the pressure dependent mechanism the results seem to indicate that the variation with pressure of $\frac{K_d}{K_i}$ is the result of a variation with pressure of Kd, as Burhop's theory would indicate, rather than a variation with pressure of Ki. With Burhop let us assume that the sole variation with pressure takes place in Kd. The ionization cross section Ki is assumed
Figure I-10  $\alpha$ Particle Ionization in He - $N_2$ Mixtures

Figure I-11  Dependence of $\frac{K_d}{K_i}$ on Pressure for Mixtures of $A$, $H_2$ and $N_2$ in He
independent of pressure and variable only from gas to gas of the contaminant. With these assumptions the three plots of the equation $\frac{K_d}{K_1} = c_p + c'$ shown in Figure I-11 should be identical except for the ordinate scale.

In Figure I-11 the plots for $H_2$ in $He$ and $N_2$ in $He$ have been normalized to coincide with the intercept on the ordinate axis for $A$ in $He$. This is accomplished by multiplying each ordinate value in the individual plot by a constant factor. The normalized plot for $N_2$ is shown as a dotted line falling just below the $A$ plot. The normalized plot for $H_2$ is practically in coincidence with the original $A$ line. So close indeed is the agreement that no separate distinct dotted line can be drawn for the normalized $H_2$ plot.

The exact agreement here encountered for $H_2$ is largely illusory, since the three points in the original plot do not uniquely define any particular line to the exclusion of all others. The relatively poor measurements for $H_2$ are quite understandable, since its cross section for ionization is small in comparison with those of gaseous impurities coming off the walls of the chamber.

Even if one discounts the accuracy of the $H_2$ measurements, the very good agreement between $N_2$ and $A$ would seem to indicate the correctness of the suggestion of Burhop as to the deactivation of metastable helium atoms by the formation of helium molecules.

A most interesting fact connected with the pressure effect has been observed. This is that the pressure effect observed for $A$, $N_2$ and $H_2$ as contaminants disappears when $Kr$, $CO_2$, $Xe$ and $C_2H_4$ are used as contaminants. In this connection the following table is quite suggestive.

**TABLE I-7**

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Pressure Effect</th>
<th>Ionization Potential of Contaminant Gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>+</td>
<td>15.76 ev</td>
</tr>
<tr>
<td>$N_2$</td>
<td>+</td>
<td>15.51</td>
</tr>
<tr>
<td>$H_2$</td>
<td>+</td>
<td>15.43</td>
</tr>
<tr>
<td>$Kr$</td>
<td>0</td>
<td>14.0</td>
</tr>
<tr>
<td>$CO_2$</td>
<td>0</td>
<td>13.73</td>
</tr>
<tr>
<td>$Xe$</td>
<td>0</td>
<td>12.127</td>
</tr>
<tr>
<td>$C_2H_4$</td>
<td>0</td>
<td>10.5</td>
</tr>
</tbody>
</table>
While as yet no completely coherent theory has been derived, it seems plausible to assume that the excited \( \text{He}_2 \) molecules can ionize the impurity molecules directly, when the ionization potential of the latter is somewhat less than 15 ev.

In the idealized case discussed in Reference 1 the competition between radiation processes and processes involving ionization of an impurity by metastable atoms was taken up. In each of these competitive processes the probability of the reaction was proportional to the pressure, and hence the ratio \( \frac{K_d}{K_i} \) was constant and independent of pressure.

In the case where the formation of \( \text{He}_2 \) molecules takes place, the probability of this reaction depends upon the square of the pressure, or the cross section \( K_d \) upon the first power of the pressure. The ionization potential of the admixed impurity may be too high for a direct transfer of energy from the \( \text{He}_2 \) excited molecule to a molecule of the impurity with the formation of a pair of ions. The only method then of deactivation of the \( \text{He}_2 \) excited molecule is one involving solely radiation processes. With the competitive ionization process removed, \( \frac{K_d}{K_i} \) should rise in proportion to the pressure as is observed.

When, however, the ionization potential of the gaseous impurity lies below the net energy available in the excited \( \text{He}_2 \) molecule, it seems reasonable to suppose that a direct transfer of energy can take place with the production of an ion pair. This then restores the competitive process and thus tends to annul the pressure effect.

It is interesting to note that other estimates\(^{(8)}\) from spectroscopic data of the energy release, when an excited \( \text{He}_2 \) molecule goes to the ground state and dissociates, are in agreement with the approximate value of 15 ev found here.
REFERENCES


DRIFT VELOCITY OF ELECTRONS IN GASES

Joseph C. Bowe

Introduction

A parameter of interest in the conduction of electrons through a gas is the value of the average velocity with which they move in the direction of the applied field. This information has immediate practical usefulness in the design of fast, gas-filled counting chambers. In addition, as pointed out by Nielsen,\(^1\) accurate curves of the variation of electron drift velocity with the applied electric field should provide a test of the theories of electron collision, scattering and energy transfer for very low electron energies in the range of 0-10 volts. Indeed, direct experimental observation of these quantities by other methods would be extremely difficult or impossible at such low energies.

The data existing in the literature are restricted to measurements made in only a few different gases and gas mixtures. Results of several workers\(^1,2,3,4,5\) who have made measurements in argon are shown in Figure 1-12. The discrepancies have been ascribed to the differences in gas purity.\(^5\) We are planning to repeat the measurements which have been published by other workers and then use a variety of gases and gas mixtures of special interest.

Method of Measurement

A group of photoelectrons is released from the cathode of a parallel plate chamber and the time of transit across the gap is measured by observing on the oscilloscope the length of the signal due to their displacement. The number of photoelectrons released in each burst is large enough to permit the use of a wide band amplifier and the observation on the oscilloscope of the current pulse in the chamber. Ideally, the current pulse, as viewed on the oscilloscope, should be a signal with sharp edges and of constant amplitude, the duration of which corresponds to the electron transit time. In practice, the leading edge has a rise time due to the fact that all the photoelectrons are not released instantaneously. The trailing edge has a longer decay time which is longer due to the diffusion of the electrons. By increasing the time constant of the input circuit, the current can be integrated, in which case, a voltage pulse whose rise time corresponds to the transit time of the electrons is seen on the scope. Since a larger signal is obtained this way, less electronic amplification is required. However, observation of the current pulse has the advantage that one can “follow” the electrons in their flight across the chamber. Oscillograms of current and voltage pulses are shown in Figure 1-17.
Figure I-12

DRIFT VELOCITY OF ELECTRONS IN ARGON

KLEMA & ALLEN (1950)

TOWNSEND & BAILEY (1922)

KIRSHNER & TOFFOLO (1952)

COLLI & FACCHINI (1952)

NIELSEN (1936)
The transit times can be measured very rapidly by visual observations of the oscilloscope trace, or a photograph can be taken and scrutinized later. The drift velocity is obtained by simply dividing the separation of the parallel plates by the transit time.

**Apparatus**

The electronic equipment consists of a high voltage supply for the flash tube, a manually operated triggering unit to initiate the flash and a voltage supply for the parallel plate chamber. The signal is amplified with a Tektronix pre-amplifier type 121 and scope 511AD. This combination has a maximum gain of $10^4$ and a bandwidth of 8 mc. The chamber is evacuated by a mechanical pump to about 10 microns. A mercury manometer is used for pressure measurement. The gas is purified by a hot calcium convection circulator which is attached to the chamber. A schematic arrangement of the equipment is shown in Figure I-13.

The ultraviolet light source consists of a special flash tube developed for us by the Kemlite Laboratories.* In order that the light source be satisfactory for this experiment, it is necessary that the time duration of the flash be small compared with the transit time of the electrons and secondly, that the light be sufficiently intense in the ultra-violet to release a large number of photoelectrons from the cathode. Both of these requirements have been met.

Much of the apparatus is in the process of being rebuilt. The vacuum system in the new arrangement has an oil diffusion pump, a Wallace and Tiernan precision dial manometer replaces the mercury manometer, a helium-tight, all metal valve is used on the chamber and soft metal gaskets and soldered connections are used throughout. A forced circulating system is installed for the purpose of gas purification.

**The Ionization Chamber**

A diagram of the parallel plate chamber now in the process of construction is shown in Figure I-14. The cathode is a disk of flat plate glass, 7-1/2 inches in diameter by 1/4 inch in thickness which is made conducting and photosensitive by a coating of tin. The anode consists of a brass disk, 3 inches in diameter by 1/8 inch in thickness. A perforated area covering about 1 square inch and having 30 per cent transmission is located in the center of the anode to allow the passage of light to the cathode. The anode-guard ring assembly is mounted on three insulating supports. The cathode is supported by and insulated from three brass stand-offs. The distance between the plates is 1 inch, but it can be easily increased or decreased. A quartz window 1-3/8 inches in diameter by 1 mm in thickness is mounted on the housing above the anode. Electrical connections are brought out by glass Kovar feed-throughs. All other insulators in the chamber are made

*1819 W. Grand Avenue, Chicago 22, Illinois
Figure I-13
Figure 1-14
of Kel-F. A stainless steel chamber containing calcium turnings which can be heated to any desired temperature is attached to the chamber. Positive circulation of the gas is achieved with a sylphon bellows pump.

Special precautions are taken to eliminate possible contamination of the gas due to leakage from the atmosphere or outgassing of materials in the chamber. Since teflon and Kel-F were found to be porous to helium, they are not used as gaskets, feed-through insulators or as the shut-off disk in the valves connected to the chamber. Soft metal gaskets are used throughout. Kel-F is tolerated in the chamber because of its reputed low vapor pressure. Valves connected to the chamber will be of all-metal construction, 5/8-inch port. A problem still to be solved is the attachment of a 1-mm thick quartz window to the brass chamber. The bond must be vacuum tight, of low vapor pressure material and capable of withstanding temperatures of at least 100°C.

Results

The results presented here are to be considered only as preliminary and indicative of the feasibility of the method. The ease and rapidity with which results can be obtained is a prominent feature of the method. Many refinements are to be introduced with the new equipment.

Observations over a period of time have been made using nitrogen and argon. In Figure I-15 we present data which was taken immediately after filling the chamber with tank argon, 99.9 per cent pure. The solid curve is the one published by Kirshner and Toffolo. The agreement is surprisingly good and unexpected due to the fact that mercury vapor is known to be present in our chamber, that teflon gaskets were employed, and that lucite, which is believed to have a rather high vapor pressure, was used as supports for the anode. In addition, the scope was self-triggering and the sweep speeds were not calibrated. The measurements were made by visual observation of the oscilloscope trace. A second curve which was taken after the chamber was allowed to stand for three days is shown in Figure I-16. The shape of the curve resembles that of Klema and Allen and illustrates the effects of contamination due to outgassing, leakage, etc. The hot calcium convection purifier was in continuous operation at 420°C. Typical oscillograms of the current and voltage pulses obtained in argon are shown in Figure I-17. Each photograph represents about 30 traces.
Figure I-15  Experimental Data on the Drift Velocity of Electrons in Argon

The Solid Curve is that Published by Kirshner and Toffolo

Figure I-16  Experimental Data on the Drift Velocity of Electrons in Argon Taken a Few Days after Data in Figure I-15
FIGURE I-17  TYPICAL CURRENT AND CORRESPONDING VOLTAGE PULSES
(SHOWN SIDE BY SIDE FOR COMPARISON)

Chamber Voltage: A and C, 300 Volts - B and D, 600 Volts
Amplifier Gain: A and C, Current, $7 \times 10^3$ - Voltage, 90
B and D, Current, $3 \times 10^3$ - Voltage, 40
APPENDIX I

Release of Free Electrons in a Chamber: Experimental Details

J. C. Bowe

In recent years, a popular method of obtaining free electrons in a gas-filled chamber for the purpose of measuring drift velocities has been the use of a radioactive alpha emitter. Because of the relatively small signal obtained from an alpha-particle, a high gain pulse amplifier is required for observation and measurement. Consequently, only voltage pulses can be viewed on the oscilloscope. Another restriction which accompanies the use of alpha-particles is the limitation in gas pressures that can be used. The range of the alpha-particle increases as the gas pressure is reduced and it may finally exceed the uniform field region of the chamber at low pressure. In addition, the pulse height depends upon the track orientation unless a gridded chamber is used.

Hudson(6) developed a method in which volume ionization was produced by a burst of soft x-rays. The data obtained in this manner require an analysis involving successive approximations.

The present experiments were initiated under the direction of Dr. Francis R. Shonka,* former Director of the Division of Instrument Research and Development, who originally proposed the idea of using a pulsing light source to release bursts of photoelectrons from the cathode of a gas-filled chamber. Some time later, it came to our attention that Hornbeck(7) was using this idea in his studies of the Townsend discharge and had demonstrated its applicability to the measurement of electron drift velocity. The advantage of this method is the possibility of releasing large numbers of electrons in each burst so that relatively large signals are obtained from the chamber. The signals can be amplified with a wide band transient amplifier permitting observation of the current pulse. In this way, one can "follow" the electrons as they cross the chamber. Also, all the electrons in the cloud are initially of very low energy and there is no doubt concerning their point of origin.

The problems of finding a suitable light source and photosensitive surface are complementary. Sensitive surfaces which require very special preparation, treatment and handling could not be used because there is no way of conveniently depositing a fresh surface in our chamber or of keeping it clean. A number of ordinary untreated metals were compared for their photoelectric emission when illuminated with a germicidal lamp. Tin was found to give the highest yield and to be the most constant over a period of time. Subsequently, the cathode was made from a piece of flat pyrex plate glass upon which a coating of tin was evaporated.

* Present Address: Physical Sciences Laboratory, St. Procopius College, Lisle, Illinois.
The problem of obtaining a short flash of light involves the tube design, gas filling and associated circuitry. The requirements placed on the light source are that it be capable of giving a pulse of light whose time duration is short compared with the transit time of the electrons and whose ultraviolet content is sufficient to release a large number of electrons from the cathode. A flash lamp which would meet these requirements was not commercially available. The Kemlite Laboratories became interested in this problem and have developed a satisfactory tube for us. The tube is constructed of 7-mm quartz tubing with an electrode separation of 1-1/2 inches and has a holdoff voltage in the neighborhood of 8000 volts. Flashing is accomplished by discharging a condenser through it. If the condenser is charged to a voltage below the hold-off, the tube may be fired at will by means of a manually operated trigger circuit. By discharging a 0.01-mfd condenser through the tube, a pulse of 0.2 microsecond duration was obtained from the evacuated chamber.
APPENDIX II

A Gastight Chamber

Joseph C. Bowe and Eugene A. Mroz

If the drift velocity of electrons in argon is plotted against values of \( E/p \), it is known that the form and shape of the resulting curve depends upon the purity of the gas. In the case of nitrogen, the purity of the gas is apparently of no consequence as is evidenced by the agreement to be found in the literature. In the present experiments, an attempt is being made to eliminate contamination of the gas due to outgassing of organic matter in the chamber or due to leakage through gaskets or valves. We have made a number of tests on teflon and fluorothene with the helium leak detector and are convinced that these substances are porous to helium. This precluded their use as gaskets, electrical feed-through insulators or as shut-off disks in valves. All gaskets used in the chamber are made of soft metal.

In our search for a gastight, all metal valve, nothing satisfactory was to be found on the commercial market. We have tested some all metal needle valves and found them to be helium-tight. But these valves have a 1/8-inch port, whereas a larger port, say 5/8 inch, was desired. The aid of the members of our staff shop was engaged in an attempt to construct a gastight all metal valve. In the first approach, a Veeco bellows type valve was modified by replacing the shutoff teflon disk with a 3/4-inch steel ball bearing which had been lapped with the seat. A larger handle was placed on the valve so that greater pressure could be applied between the ball bearing surface and the seat. One side of the valve was connected to the helium leak detector while the other side was exposed to a helium atmosphere. The valve was found to be tight for a few hours before it suddenly developed a small leak. The cause of the leak was not determined, but turning the handle tighter would sometimes stop the leak temporarily. A second Veeco valve was modified by replacing the brass seat with one made of stainless steel and the teflon disk by one of soft metal, such as copper or 2S aluminum. This valve remained helium-tight over the period of time that we checked it. A third valve was found to be equally tight when only the teflon disk was replaced by one made of soft metal. Diagrams of these valves are shown in Figure I-18. We do not as yet have a history of experience with them, but they look very promising.

We are still confronted with the problem of attaching the 1-mm quartz window to the chamber in such a way that the resulting bond will be gastight, capable of being heated to at least 100°C and able to withstand a differential pressure of 1 atmosphere. A vacuum-tight seal was achieved with a bonding resin, but it was not used because of the possibility of contaminating the chamber gas. Lead-indium solder will wet quartz, but we have not been successful in obtaining a gastight seal with it.
Figure I-18
FULL SIZE
REFERENCES


A special ion tube and its auxiliary equipment is now ready for initial tests without halogens. Its design and construction has been undertaken to investigate the role of the alkali-sensitized emitting surface in the operation of the halogen-sensitive positive ion emitter in air at atmospheric pressure. (See ANL-4948, H. A. Schultz and J. G. Dodd, Jr., pp. 109-129.)

The chlorine dilution system has been constructed in sections in order to allow its connection with little interruption of tests on the rest of the equipment.
Experimental Meteorology Stack Studies

A series of smoke measurements were made on thirteen different days with the Experimental Stack described previously. During the course of the experiments a variety of wind speeds, wind directions, and stability conditions prevailed. These smoke diffusion measurements, made under many different meteorological conditions, will be useful in determining quantitatively the effects of weather parameters on atmospheric diffusion. Chemical Corps Smoke Pots, type HC-1, were used on all of the runs and the smoke-air mixture was ejected from the stack at rates ranging from 500 to 4300 cubic feet per minute.

Photogrammetric measurements of the smoke plume were made during each series of experiments. Simultaneous pictures by two cameras were taken every 5 seconds on all but the last two runs; on the latter, picture pairs were obtained every 3-1/2 seconds.

Improvements

As one usually finds with a new piece of equipment a number of improvements are desirable or even necessary. During this period the following improvements were made on the Experimental Meteorology Stack and the auxiliary equipment:

Improvement on Hagan Flow Meter Mounting

It was found that the Hagan Flowmeter used in measuring the flow of the air-smoke mixture through the stack would not remain in a level position with the mounting used. The meter was mounted on a 2-in. iron pipe driven 3 ft into the ground. To overcome this difficulty the iron pipe was placed in a concrete block.

Design of a New Smoke Injection Scheme for Use with Smoke Generator

A method for injecting the output of the smoke generator directly into the stack has been designed. This will incorporate a more efficient technique for producing smoke puffs. It was necessary to design this equipment since the smoke intake capacity of the stack is too low and smoke pots are messy to handle. After about 5 or 6 series of runs as much as 1 inch of debris may be found in the ductwork. This debris is acidic and can cause damage to the ductworks.
**Use of Polaroid Lens Filters**

A number of filters for improving the contrast between the smoke and the background were tested. Polaroid filters were found to be best and are now being used.

**Improvement in Film Analysis Techniques**

A light table was obtained and a film winder for use with the light table was designed and built. With this apparatus it is possible to view two rolls of film simultaneously. The film winder allows the winding of either one roll of film separately or both rolls together. A photograph of this apparatus is shown in Figure I-19.

A special grid was incorporated in the K-24 cameras used with the Experimental Meteorology Stack. The grid is photographed each time a frame is shot and therefore appears on the photograph. A sample photograph with the grid superimposed is shown in Figure I-20. The spacing of the grid lines was calculated to allow the determination of the true position in space of a point identified on each of the two photographs taken simultaneously.

**Calibration Checks**

A check was made on our photogrammetric technique for determining the true position of points in space. This was done by setting up a group of markers in space and determining their positions by two independent methods: (1) the photogrammetric technique and (2) the double transit method. The two measurements checked satisfactorily.

To suspend markers in space, four approximately parallel nylon cords were strung between the Experimental Meteorology Stack and the Meteorology Tower - a distance of 225 feet. The uppermost cord was located about 110 feet and the lowest about 40 feet above the ground. The other two were evenly spaced between these. Seven or eight yellow markers, 4 in. x 4 in. x 5-1/2 in., were attached and distributed along each cord.

A 400-foot base line was used for making both the double transit and photogrammetric measurements. A second set of photogrammetric measurements was made from another 400-foot base line which differed in orientation by 20°. These 400-foot base lines were part of a group of carefully surveyed base lines used for making the photogrammetric measurements.

**Stability Measurements**

The thermodynamic stability of the lower layers of the atmosphere is an important factor in determining the rate of atmospheric diffusion. For the past three years we have been obtaining data on stability by measuring the difference in temperature between 144 feet and 5-1/2 feet above the ground.
Figure 1-19  Light Table and Film Winding Apparatus for Analyzing Diffusion Study Photographs
Figure 1-20  A Diffusion Study Photograph using HC-1 Smoke taken by a K-24 Camera with Incorporated Evaluation Grid, Experimental Meteorology Stack located at Meteorology Laboratory.
This is not entirely adequate since the variation of temperature with height is a non-linear function. For this reason special booms for mounting thermocouples have been designed to obtain temperature measurements at the intermediate levels of 18.75, 37.5, and 75 feet above the ground. The design, fabrication, and installation of these booms were completed within this period.

These booms consist of 3-inch aluminum tubes 15 feet 10 inches long to which are attached an Eastern Air Devices type 57C suction fan. The booms are mounted so that the temperature measuring element is placed immediately below the anemometer located at each of these levels. Photographs of these booms are shown in Figures I-21 and I-22.

Up to now we have used a copper-constantan thermopile consisting of 5 thermocouples as the temperature measuring element. With the installation of this system single thermocouples will be used at all levels. The adoption of a single-thermocouple measuring element results in a considerable economy since the 5 sets of thermocouple lead wires already running to the top of the tower in conduit will be used with the single thermocouples at each of the intermediate levels.

New recorders will not be necessary for the additional measurements. It is planned to program the information so that a complete temperature sounding will be made about every 10 minutes. The fabrication of the programming equipment has not yet been completed.

The Electronic Wind Vane

No further work on the development of the Electronic Wind Vane was performed during this six-month period. However, descriptions of the instrument were prepared for the Patent Office and the Geophysics Research Directorate of the U. S. Air Forces. The latter description is an expansion of a paper submitted to this group previously. The present paper is to be included in the publication dealing with the instrumentation used on the Great Plains Turbulence Field Program carried out at O'Neill, Nebraska, during the summer of 1953.

Climatology

ANL-5256 dealing with climatological measurements taken at Argonne from July, 1952 to June, 1953 was completed and distributed.

The groundwork for the 5-year climatological summary based on measurements taken here at Argonne and data from the U. S. Weather Bureau offices at Chicago and Joliet was laid.
Figure I-21  Temperature and Wind Booms at 19 foot level on Meteorology Tower
Figure I-22  Temperature and Wind Booms on Meteorology Tower
An outline for this forthcoming summary was prepared after consultation with many other meteorologists and examination of a number of analogous studies.

The data have been placed on IBM punch cards and the analysis will be carried out by machine techniques. This work is now in progress.

**Technical Services**

The Meteorology Group was requested to furnish advice on a number of special problems; some of these are: (a) Atmospheric diffusion pertaining to the burning of NaK under certain conditions, (b) Atmospheric pressure variations in connection with hood design work in the proposed metals laboratory, (c) Hail forecasting to protect the Laboratory's greenhouses.

The Meteorology Group was host for one afternoon to a group of 35 Weather Reserve Officers on a two-week tour of duty. Lectures were presented on (1) atmospheric diffusion, (2) use of IBM machine techniques in handling weather data, and (3) the specialized instrumentation of the Meteorology Laboratory.

The Meteorology Group has accepted an invitation of the U. S. Weather Bureau to join the U. S. Solar Radiation network. This involves furnishing the U. S. Weather Bureau with daily totals of solar radiation measured at Argonne for inclusion in a monthly publication summarizing the weather over the U. S.

**REFERENCE**

MISCELLANEOUS ACTIVITIES

The division has made available to Dr. H. H. Rossi of Columbia University various neutron sources (D-D, Pu-Be) and the multichannel pulse height analyzer and cooperated with him in the collection of data pertinent to his L.E.T. meter. Results will be published by Dr. Rossi.

(J. E. Rose and C. E. Miller)

The primary circuit of the Westinghouse Industrial Type 250 kv x-ray machine installed in our calibration facility has been modified in order to obtain fine primary voltage control over a range of 4 volts. This range covers two steps of the manufacturers' fine step control, and it is operative throughout the voltage range available to the primary circuit. The original voltmeter in the machine is retained as indicator, and the original kv-P.V. calibration of the machine is remains unchanged by the modification. The fine adjustment is obtained by inserting in series with the mains, the secondaries of two 5 v - 40 amp filament transformers connected in parallel (with due attention to polarity!). The primaries of these transformers are fed by a 0-135 v, 3 amp autotransformer (Variac) properly phased to the 110 v ac line.

(S. S. Brar and J. E. Rose)

A C.P. high voltage supply (30 kv maximum at 100 ma) designed to supply the power for x-ray tubes used in microradiography has been delivered and tested.

When used in conjunction with a Machlett A-2 x-ray tube and a completely redesigned microradiographic camera, a test series of exposures of a 70-micron thick section of human bone yielded microradiographs of excellent quality.

(R. E. Rowland)
PUBLICATIONS

July 1, 1954 through December 31, 1954


Moses, H., and G. R. Hilst. The Electronic Wind Vane. (Submitted to Geophysics Research Directorate to be published in a special volume.)

PART II

QUARTERLY REPORT OF BIOLOGICAL AND MEDICAL RESEARCH DIVISION

FOR

OCTOBER, NOVEMBER, DECEMBER 1954

A. M. Brues
Director
# PART II

## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular permeability and Krebs 2 ascites tumor</td>
<td>71</td>
</tr>
<tr>
<td>R. L. Straube and M. S. Hill</td>
<td></td>
</tr>
<tr>
<td>Growth of the Krebs 2 ascites tumor in partially hepatectomized mice</td>
<td>71</td>
</tr>
<tr>
<td>R. L. Straube and M. S. Hill</td>
<td></td>
</tr>
<tr>
<td>Radiation effects on tumor nuclei</td>
<td>72</td>
</tr>
<tr>
<td>A. M. Brues and A. N. Stroud</td>
<td></td>
</tr>
<tr>
<td>Simultaneous administration of Ra$^{226}$ and Ca$^{45}$ to dogs</td>
<td>74</td>
</tr>
<tr>
<td>W. P. Norris, T. W. Speckman and B. J. Holloway</td>
<td></td>
</tr>
<tr>
<td>Effect of surgical trauma on survival following low-rate X irradiation</td>
<td>76</td>
</tr>
<tr>
<td>S. P. Stearner, M. Sanderson and E. J. Christian</td>
<td></td>
</tr>
<tr>
<td>Urea formation in irradiated nephrectomized rats. II.</td>
<td>79</td>
</tr>
<tr>
<td>J. F. Thomson and E. M. Moss</td>
<td></td>
</tr>
<tr>
<td>The effect of X-irradiation on the dry weight and protein content in regressing embryos</td>
<td>81</td>
</tr>
<tr>
<td>T. N. Tahmisian, B. J. Wright and R. L. Devine</td>
<td></td>
</tr>
<tr>
<td>Physical fitness and acute radiation lethality in mice</td>
<td>83</td>
</tr>
<tr>
<td>R. L. Straube and H. M. Patt</td>
<td></td>
</tr>
<tr>
<td>Low-energy X ray phantom dosimetry</td>
<td>84</td>
</tr>
<tr>
<td>H. Walton, Jr.</td>
<td></td>
</tr>
<tr>
<td>The gamma ray toxicity program: Calibration and equipment</td>
<td>91</td>
</tr>
<tr>
<td>G. A. Sacher, D. Grahn, S. Lesher and K. Hamilton</td>
<td></td>
</tr>
<tr>
<td>Recovery and regeneration of spleens after single and divided doses of X-irradiation</td>
<td>95</td>
</tr>
<tr>
<td>A. N. Stroud</td>
<td></td>
</tr>
<tr>
<td>Pilot study on irradiation of biological materials and carcinogenicity</td>
<td>100</td>
</tr>
<tr>
<td>A. M. Brues, K. A. Hamilton and H. Walton, Jr.</td>
<td></td>
</tr>
<tr>
<td>Radio-ruthenium toxicity studies</td>
<td>102</td>
</tr>
<tr>
<td>H. Walton, Jr. and A. M. Brues</td>
<td></td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity of Sr$^{89}$ in mice. Malignant bone tumors</td>
<td>106</td>
</tr>
<tr>
<td>M. P. Finkel, H. Lisco and A. M. Brues</td>
<td></td>
</tr>
<tr>
<td>The daily probability of death in fast neutron- and Co$^{60}$ gamma-</td>
<td>118</td>
</tr>
<tr>
<td>irradiated CF # 1 female mice</td>
<td></td>
</tr>
<tr>
<td>H. H. Vogel, Jr., J. W. Clark and D. L. Jordan</td>
<td></td>
</tr>
<tr>
<td>Thermal neutron contribution to a fast neutron exposure</td>
<td>121</td>
</tr>
<tr>
<td>H. Walton, Jr.</td>
<td></td>
</tr>
<tr>
<td>Influence of the hypophysis and of the adrenal cortex upon the</td>
<td>123</td>
</tr>
<tr>
<td>tissue mast cell of the hamster</td>
<td></td>
</tr>
<tr>
<td>D. E. Smith, Y. S. Lewis and S. T. Hartig</td>
<td></td>
</tr>
<tr>
<td>Pyresis studies</td>
<td>125</td>
</tr>
<tr>
<td>M. W. Rosenthal and J. F. Fried</td>
<td></td>
</tr>
<tr>
<td>Method for the preparation of 5'-methyl- and 5'-ethylthioadenosine</td>
<td>126</td>
</tr>
<tr>
<td>F. Schlenk</td>
<td></td>
</tr>
<tr>
<td>Indole-3-acetic acid oxidase</td>
<td>128</td>
</tr>
<tr>
<td>R. E. Stutz</td>
<td></td>
</tr>
<tr>
<td>The behavior of ribonuclease in strong acid media. Biological and</td>
<td>133</td>
</tr>
<tr>
<td>physical properties of recovered material</td>
<td></td>
</tr>
<tr>
<td>A. L. Koch, W. A. Lamont and J. J. Katz</td>
<td></td>
</tr>
<tr>
<td>Distribution of isotope in the purines of <em>Escherichia coli</em> after</td>
<td>140</td>
</tr>
<tr>
<td>growth in the presence of various radioactive precursors</td>
<td></td>
</tr>
<tr>
<td>A. L. Koch</td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic localization of the tryptophan-indoleacetic acid</td>
<td>145</td>
</tr>
<tr>
<td>enzyme system</td>
<td></td>
</tr>
<tr>
<td>S. A. Gordon, A. L. Koch and W. A. Lamont</td>
<td></td>
</tr>
<tr>
<td>The stability of DNA during growth</td>
<td>148</td>
</tr>
<tr>
<td>R. W. Swick, D. M. Tahara and A. L. Koch</td>
<td></td>
</tr>
<tr>
<td>Tryptophan peroxidase and arginase in regenerating liver</td>
<td>150</td>
</tr>
<tr>
<td>J. F. Thomson and E. M. Moss</td>
<td></td>
</tr>
<tr>
<td>Hemolysin production in X-irradiated rabbits</td>
<td>152</td>
</tr>
<tr>
<td>B. N. Jaroslow and W. H. Taliaferro</td>
<td></td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>The relationships between nucleolar and nuclear growth and form during development in Paramecium</td>
<td>152</td>
</tr>
<tr>
<td>C. F. Ehret and E. L. Powers</td>
<td></td>
</tr>
<tr>
<td>Cleavage time in X-irradiated <em>Ascaris</em> eggs</td>
<td>154</td>
</tr>
<tr>
<td>T. N. Tahmisian, R. L. Devine and B. J. Wright</td>
<td></td>
</tr>
<tr>
<td>Studies on the role of the nucleus in protein synthesis</td>
<td>155</td>
</tr>
<tr>
<td>A. D. Barton and A. K. Laird</td>
<td></td>
</tr>
<tr>
<td>Effects of X ray on fertility in the mouse</td>
<td>156</td>
</tr>
<tr>
<td>M. Sanderson and S. P. Stearner</td>
<td></td>
</tr>
<tr>
<td>Effect of temperature on phosphate release</td>
<td>157</td>
</tr>
<tr>
<td>J. M. Ginski</td>
<td></td>
</tr>
<tr>
<td>The kinetics of glycine incorporation by <em>Escherichia coli</em> during exponential growth</td>
<td>159</td>
</tr>
<tr>
<td>A. L. Koch</td>
<td></td>
</tr>
<tr>
<td>A comparison of the rate of uptake of 2-Cl4-glycine by bacteria in various phases of growth</td>
<td>162</td>
</tr>
<tr>
<td>A. L. Koch</td>
<td></td>
</tr>
<tr>
<td>Application of leucocyte antiserum to study of radiation-induced leucopenia</td>
<td>167</td>
</tr>
<tr>
<td>H. M. Patt, M. A. Maloney and E. M. Jackson</td>
<td></td>
</tr>
<tr>
<td>Recovery of blood leucocytes after acute peripheral depletion</td>
<td>168</td>
</tr>
<tr>
<td>H. M. Patt, M. A. Maloney and E. M. Jackson</td>
<td></td>
</tr>
<tr>
<td>The use of plastics in the preparation of thin sections of undecalcified bone; with special reference to radioautographic procedures</td>
<td>170</td>
</tr>
<tr>
<td>L. A. Woodruff and W. P. Norris</td>
<td></td>
</tr>
<tr>
<td>Preliminary experiments on the mechanism of spleen protection</td>
<td>173</td>
</tr>
<tr>
<td>H. S. Ducoff</td>
<td></td>
</tr>
<tr>
<td>The effect of serum, plasma proteins, and other substances on the survival of ascites tumor cells after X-irradiation</td>
<td>176</td>
</tr>
<tr>
<td>A. N. Stroud and A. M. Brues</td>
<td></td>
</tr>
<tr>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>The effect of fluoroacetate administration on experimental plumbism</td>
<td>178</td>
</tr>
<tr>
<td>J. F. Fried and M. W. Rosenthal</td>
<td></td>
</tr>
<tr>
<td>Statistical analysis of neutrophil, lymphocyte, and erythrocyte</td>
<td>179</td>
</tr>
<tr>
<td>counts from a normal human population</td>
<td></td>
</tr>
<tr>
<td>J. M. Gurian and G. A. Sacher</td>
<td></td>
</tr>
<tr>
<td>Murine mange control program</td>
<td>182</td>
</tr>
<tr>
<td>R. J. Flynn and B. N. Jaroslow</td>
<td></td>
</tr>
</tbody>
</table>
VASCULAR PERMEABILITY AND KREBS 2 ASCITES TUMOR GROWTH

R. L. Straube and M. S. Hill

Altered vascular permeability may be a factor contributing to the accumulation of peritoneal fluid during growth of ascites tumor cells. By means of intravenous injections of I\textsuperscript{131}-labeled human serum albumin we have shown that: (1) the escape of radioactive material from blood to peritoneal cavity is greater than normal in the ascites tumor-bearing animal and (2) the larger the inoculum of tumor cells, the greater the activity which accumulates in an equivalent period. Only 2\% of the injected activity is found in the peritoneal cavity of a normal mouse two hours later. In contrast, 5\%, 10\%, and 20\% of the injected activity are found in the abdominal cavities of mice inoculated 3 days earlier with $2 \times 10^6$, $4 \times 10^6$ and $20 \times 10^6$ cells. Present experiments are concerned with further characterization of leakage in terms of the number of viable and non-viable tumor cells during the course of tumor development.

GROWTH OF THE KREBS 2 ASCITES TUMOR IN PARTIALLY HEPATECTOMIZED MICE

R. L. Straube and M. S. Hill

We have observed that intraperitoneal inoculation of ascites tumor cells reduces the rate of liver regeneration after partial hepatectomy. There is also the suggestion that the reduction in rate is related to the inoculum size. Four days after partial hepatectomy, the control liver has regained 42\% of its initial weight. In contrast, liver weight is unchanged in mice inoculated with $20 \times 10^6$ cells one day after hepatectomy and has increased by only 9\% in animals injected with $2 \times 10^6$ cells. At 6 days, the respective weight increases are 82\%, 20\% and 88\%. Tumor cell growth, as evidenced by the presence of ascites, apparently occurs in the absence of a large part of the liver, although whether this growth occurs at a reduced rate has not been determined. A quantitative study of liver regeneration and tumor cell number is in progress.
RADIATION EFFECTS ON TUMOR NUCLEI

A. M. Brues and A. N. Stroud

Further microscopic studies have been made on sections of experimental tumors irradiated with point beta sources, to determine the rate and extent of nuclear enlargement. Using a camera lucida, we measured the diameters of at least 25 nuclei in several areas of four of the tumors investigated: the Flexner-Jobling rat carcinoma, Jensen rat sarcoma, Crocker mouse sarcoma 180, and Hall mouse carcinoma.

Since it was observed previously that dose rates a little less than 1 rep/min represented the borderline above which mitosis was suppressed, measurements of nuclear size were made at the point where the dose rate is of this order, estimated at 3.5 mm from a 50 μc bead, as well as at 2 mm and 6 to 7 mm. Table II-1 shows mean nuclear diameters as a function of distance (and of dose rate) several days after implantation. The dose rates are merely approximate and have not been corrected for variations in the activity of beads.

<table>
<thead>
<tr>
<th>mm distance</th>
<th>rep/min</th>
<th>rep/day</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>10</td>
<td>14,000</td>
<td>0</td>
</tr>
<tr>
<td>3.5</td>
<td>0.7</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>6 to 7</td>
<td>&gt;0.05</td>
<td>&gt;75</td>
<td>0</td>
</tr>
</tbody>
</table>

| Flexner-Jobling, 5 days | 20.0 | 19.8 | 10.0 | 10.0 |
| 8 days               | 25.8 | 13.4 | 11.3 | 11.7 |
| Sarcoma 180, 3 days  | 17.3 | 12.3 | 10.3 | 11.7 |
| 5 days               | 17.7 | 15.2 | 10.7 | 10.4 |
| Jensen Sarcoma, 7 days | 18.1 | 14.2 | 12.3 | 11.6 |
| Hall Carcinoma, 5 days | 23.2 | 14.0 | 11.8 | 11.6 |

It is seen that the borderline area is about the same (with one exception) in these tumors. It may be pointed out that if growth is occurring centrally to this area, these cells will be moving away from the bead, and therefore the dose rate to these cells is decreasing with time.

A number of measurements have also been made as a function of time after insertion of the source. In Table II-2 the relative nuclear volume is shown, calculated as ratios of the cubes of the mean measured
nuclear diameters, and adjusted so that the volume of the nucleus of the un-
irradiated tumor cell is equal to 1. These measurements were made at a
distance of 2 mm from the bead, where in all cases nuclear volume is maxi-
mal. It appears from this that the nuclei increase rather steadily until they
reach a maximum; and that this maximum varies with the particular tumor.

**TABLE II-2**

Relative Volumes of Nuclei as a Function of Time after Beginning
of Irradiation

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>7 to 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexner-Jobling</td>
<td>1.0</td>
<td>2.0</td>
<td>-</td>
<td>4.3</td>
<td>8.0</td>
<td>17.2</td>
</tr>
<tr>
<td>Sarcoma 180</td>
<td>1.0</td>
<td>1.7</td>
<td>3.6</td>
<td>3.2</td>
<td>3.4</td>
<td>-</td>
</tr>
<tr>
<td>Jensen Sarcoma</td>
<td>1.0</td>
<td>-</td>
<td>5.1</td>
<td>-</td>
<td>-</td>
<td>5.4</td>
</tr>
<tr>
<td>Hall Carcinoma</td>
<td>1.0</td>
<td>3.0</td>
<td>-</td>
<td>6.4</td>
<td>7.9</td>
<td>6.4</td>
</tr>
</tbody>
</table>

A few measurements were made, using the same technique, of the
diameters of nuclei and their nucleoli, since it was obvious that the latter
increase rather strikingly along with the nuclei. These measurements
were made in the Flexner-Jobling tumor, in which the nuclear enlargement
is greatest, and are shown in Table II-3. This indicates that the nucleolus
increases at a rate not dissimilar from that at which the nucleus enlarges,
and would suggest that a growth of several elements is taking place.

**TABLE II-3**

Flexner-Jobling Carcinoma: Diameters of Nucleus
and Nucleoli, and Ratio

<table>
<thead>
<tr>
<th>Days</th>
<th>Nucleus</th>
<th>Nucleolus</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days, 2 mm</td>
<td>22.4</td>
<td>8.4</td>
<td>0.37</td>
</tr>
<tr>
<td>7 mm</td>
<td>14.6</td>
<td>4.7</td>
<td>0.32</td>
</tr>
<tr>
<td>8 days, 2 mm</td>
<td>23.0</td>
<td>9.4</td>
<td>0.41</td>
</tr>
<tr>
<td>7 mm</td>
<td>11.5</td>
<td>4.2</td>
<td>0.36</td>
</tr>
</tbody>
</table>
SIMULTANEOUS ADMINISTRATION OF Ra\textsuperscript{226} AND Ca\textsuperscript{45} TO DOGS

W. P. Norris, T. W. Speckman and B. J. Holloway

Premixed solutions containing RaCl\textsubscript{2} and Ca\textsuperscript{45}Cl\textsubscript{2}, isotonic, and pH 3.0 were injected intravenously into 3 young (7 mo.) and 2 adult (15 mo.) male beagle dogs. Twenty-four hour excreta samples were analyzed at intervals over a period of 233 days for radium, calcium\textsuperscript{45}, and total calcium content. The resulting data showed that the excretion of both isotopes via the gut occurs with equal facility - the ratio of fecal Ra/Ca\textsuperscript{45} remaining at essentially that of the injected solution over the entire period (Figure II-1). However, the resorption of calcium by the kidney proved to be considerably greater than that of radium, so that the ratio of Ra/Ca\textsuperscript{45} in the urine varied between 2 and 7 in the individual animals. This value was ordinarily much higher during the first day following injection - in one case reaching 17.5. This initial high value is probably the result of some influence of the injected solution on the kidney.

Thus the difference in retention of radium and calcium appears to be directly related to kidney function. The more rapid loss of radium is reflected by a slow decrease in the ratio of retained Ra/Ca\textsuperscript{45} and this same slow change in the ratio is seen in results from fecal determinations. Analyses of bones from one animal showed the ratio of the retained materials to be identical to that in the stools at the time of sacrifice.

The data reveal no cause to believe that radium is not metabolized by bone in a manner quantitatively and qualitatively identical to calcium.
Figure II-1
EFFECT OF SURGICAL TRAUMA ON SURVIVAL FOLLOWING LOW-RATE X IRRADIATION

S. Phyllis Stearner, Margaret Sanderson and Emily Christian

Exposure of young chicks to 1000 r X ray at 43 r/min is followed by 60 - 75% mortality in the initial period (0 - 24 hrs); there is approximately 95% mortality at 30 days. In the chicks that die in the initial period, the rate at which hypotension develops is proportional to survival time. Individuals that survive 24 hours following exposure show only a minor fall in blood pressure (usually 20 - 25%) compared to the profound hypotension observed in non-survivors. Exposure to 1000 r at 6 r/min results in less than 5% early mortality in young chicks, and 40 - 60% mortality in 30 days.

To determine whether the relation between blood pressure and survival time following exposure at a low rate is similar to that which was found after high-rate exposure, three- to four-day chicks (white leghorn males) were exposed to 1000 r X rays at 6 r/min. Immediately after irradiation, the chicks were anesthetized with Nembutal (.028 mg/g I.P.), the femoral artery was cannulated and blood pressures were taken at intervals thereafter for a 6 hr period (mean arterial pressure, saline mercury manometer). It was soon apparent that the expected survival pattern was not being followed. Blood pressures fell rapidly and the group showed 100% mortality within 10 hours. When cannulation and determinations of blood pressure were postponed until three hours after the end of irradiation, the initial mortality was reduced to about 60%. Clearly, then, the restraint, surgical trauma, and blood loss associated with direct blood pressure determinations exerted sufficient stress upon low-rate irradiated birds to produce circulatory failure similar to that seen after high-rate exposures to the same dose. However, the radiation-induced physiological changes, which render the chick so susceptible to surgical trauma, undergo partial recovery within three hours after exposure.

The relative effect of surgical trauma, blood loss, and restraint on the early mortality has been determined by following the survival of groups in which these factors were separated. Sham operation with 6 hours restraint subjected birds to the stress of surgical trauma and restraint with no blood loss. The wound was sutured at the end of 6 hours. Sham operation with restraint for the minimum time required for surgery and suturing the incision removed the trauma of an open wound for 6 hours. Restraint alone for 6 hours involved neither surgical trauma nor blood loss but only the stress associated with struggling against confinement. The technical procedure was carried out either immediately after irradiation or three hours after exposure. All groups received Nembutal and were accompanied by X ray controls that received the same Nembutal dose at a comparable time after irradiation. Survival is shown in Table II-4 and may be compared with that which followed cannulation for blood pressure determinations.
TABLE II-4

Effect of Surgical Trauma, Blood Loss, and Restraint on Survival of Chicks Following Exposure to 1000 r X rays at 6 r/min

<table>
<thead>
<tr>
<th></th>
<th>No. animals</th>
<th>Per cent mortality</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial 0.24 hrs.</td>
<td>Later* 2-30 day</td>
<td>Total 30 day</td>
<td></td>
</tr>
<tr>
<td>Cannulation and 6 hrs restraint</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Immediately after X ray</td>
<td>19</td>
<td>100</td>
<td>-</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>(b) 3 hrs after X ray</td>
<td>19</td>
<td>57.9</td>
<td>87.5</td>
<td>94.7</td>
<td></td>
</tr>
<tr>
<td>Sham operation and 6 hrs restraint</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Immediately after X ray</td>
<td>72</td>
<td>88.9</td>
<td>28.6</td>
<td>93.0</td>
<td></td>
</tr>
<tr>
<td>(b) 3 hrs after X ray</td>
<td>37</td>
<td>37.8</td>
<td>65.2</td>
<td>78.4</td>
<td></td>
</tr>
<tr>
<td>Sham operation and ½ hr restraint</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Immediately after X ray</td>
<td>20</td>
<td>30.0</td>
<td>71.4</td>
<td>80.0</td>
<td></td>
</tr>
<tr>
<td>(b) 3 hrs after X ray</td>
<td>20</td>
<td>0</td>
<td>45.0</td>
<td>45.0</td>
<td></td>
</tr>
<tr>
<td>Restraint only for 6 hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Immediately after X ray</td>
<td>24</td>
<td>0</td>
<td>37.5</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td>(b) 3 hrs after X ray</td>
<td>22</td>
<td>4.6</td>
<td>52.4</td>
<td>54.5</td>
<td></td>
</tr>
<tr>
<td>X ray controls - Nembutal only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Immediately after X ray</td>
<td>52</td>
<td>1.9</td>
<td>41.2</td>
<td>42.3</td>
<td></td>
</tr>
<tr>
<td>(b) 3 hrs after X ray</td>
<td>36</td>
<td>0</td>
<td>50.0</td>
<td>50.0</td>
<td></td>
</tr>
</tbody>
</table>

* 2-30 day mortality calculated on the basis of survivors at the end of the first day.

Surgery alone, when performed immediately after X ray, was sufficient to increase early mortality. The additional stress of an open wound for 6 hours greatly increased the number of deaths and indeed was only slightly less traumatic than the actual determinations of blood pressure. This mortality was reduced by ½ when the surgery was performed three hours after exposure. Restraint alone did not increase the death rate. There was no apparent effect on later mortality in any group, although the number of animals in some instances was small.

It has been postulated that radiation effects in the initial post-irradiation period are related to release of toxic products of cell destruction, but the exact nature of such substances remains vague and experimental confirmation of the hypothesis is wanting. If this is true, however,
then the trauma of surgery and an open incision for 6 hours may be associated with release of additional toxic products of cell necrosis. This, together with the products of radiation-damaged cells, could bring the toxemia of the low-rate irradiated chick above a critical level. With time, the concentration of products of radiation-damaged cells may be so reduced that the critical level is less likely to be exceeded.

Incidentally, it should be noted that birds irradiated at the low rate showed the same relation between hypotension and survival time as was seen in chicks exposed at the high rate.
UREA FORMATION IN IRRADIATED NEPHRECTOMIZED RATS. II.

John F. Thomson and Eleanor M. Moss

Some time ago we reported on the effect of total body X-irradiation on the rate of increase of blood urea in bilaterally nephrectomized rats.\(^{(1)}\) In these experiments, a dose of 1000 r was administered 3-1/2 hours after nephrectomy and the urea levels measured until the death of the animal. Our experiences with the rat liver tryptophan peroxidase system, which is activated in irradiated animals through the adrenals,\(^{(2)}\) had led us to believe that increased ureagenesis might occur at the same time. However, we observed no effect of irradiation on the rate of urea production.

In view of the fact that, for 6 days following 660 r, rats are in a marked negative nitrogen balance,\(^{(3)}\) it was of interest to repeat these experiments with different time intervals between X-ray exposure and nephrectomy. Furthermore, since the nitrogen balance was more negative after 660 r than at 1000 r,\(^{(3)}\) we used lower doses of radiation.

In the experiments reported in Table II-5, rats were exposed to either 600 or 800 r at a rate of 200 r/min. At varying times after exposure, the kidneys were removed and the blood urea measured at 0, 6, 24, and, if possible, 30, 48, and 54 hours after nephrectomy (for methods, see \((1)\)). Only those animals which survived 24 hours or more are included. The data show that at no time was there a marked effect on urea production, although the increases seen 4 days after 800 r and 5 days after 600 r are suggestive. It might be pointed out here that the life span under these conditions is rather short; almost half of the animals died within 24 hours after removal of the kidneys.

The differences between the two control groups are probably attributable to the fact that those in the 600 r series were Sprague-Dawley females with an average weight of 269 gm., while the 800 r controls were Holtzman females weighing 224 gm.
TABLE II-5
Rate of Urea Formation in Irradiated Nephrectomized Rats

<table>
<thead>
<tr>
<th>Days after Irradiation</th>
<th>Mg. urea/hr./100 g. rat</th>
<th>600 r</th>
<th>800 r</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. rats</td>
<td>Average</td>
<td>No. rats</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>2.24 ± 0.33*</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2.16, 1.98</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>2.10 ± 0.40</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>2.25 ± 0.16</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>2.51 ± 0.27</td>
<td>-</td>
</tr>
</tbody>
</table>

*Standard deviation.

REFERENCES


THE EFFECT OF X-IRRADIATION ON THE DRY WEIGHT AND PROTEIN CONTENT OF REGRESSING EMBRYOS

T. N. Tahmisian, B. J. Wright and R. L. Devine

In previous communications\(^1\,\,2\,\,3\) it was shown that diapause embryos subjected to a dose of 25,000 r undergo a diminution in size termed "negative growth." In these embryos every nucleus becomes pycnotic, the respiratory rate increases and there is an increase in hydroquinone oxidase. In the above communications evidence was brought forth that tissue differentiation is more susceptible to X-irradiation than cell division. Cell division, in turn, is more susceptible to irradiation than the anabolic processes and the latter are more susceptible than the catabolic processes. We undertook this experiment to determine the effects of irradiation on the dry weight of the embryo and its protein content as the embryo regresses in size.

Twenty-eight-day-old diapause eggs were cleaned, dechorionated and rinsed.\(^4\) The eggs were then divided into two groups, control and experimental. The experimental group was given an X-ray dose of 25,000 r at a dose rate ranging from 1108 to 1323 r per minute. The radiation factors were 250 kv, 15 ma, without filter except for the cover (1 mm. in thickness) of a 5 cm. Petri dish.

On the day of irradiation 10 control embryos were dissected out in Bëlär's solution, rinsed first in Belär's solution to remove all of the adhering yolk, and then rinsed twice in distilled water. Each embryo was placed in 1 cc. of water, treated with the Folin phenol reagent,\(^5\) and the resulting color read in the Klett-Summerson colorimeter to determine the initial protein content. In like manner 100 embryos were dissected out, put in a tared crucible, placed in a drying oven at 125°C for 24 hours, and then weighed to determine the dry weight of the embryos. The protein and dry weight determinations were repeated at 16 and 28 days post-irradiation for both the controls and the experimentals. The whole experiment has been repeated 15 times.

The table of results shows that the dry weight as well as the protein content drops during "negative growth" and the percentage decrease in both dry weight and protein is approximately the same.

These data in conjunction with the previously-mentioned evidence suggest that 25,000 r does not destroy proteolytic enzymes. It also appears that the regression in size is not shrinkage due to protein denaturation, and that all substances are being used up at the same rate as protein is destroyed.

We wish to thank Miss Joan Gurian for the statistical analyses.
TABLE II-6

Dry Weight and Protein Content of Control and Irradiated Embryos
(Dry Weight and Protein Expressed as mg/100 Embryos)

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>X-ray</th>
<th>Control/ X-ray*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>Dry weight</td>
<td>9.320 ± 0.249</td>
<td>8.045 ± 0.135</td>
<td>1.135 ± 0.021</td>
</tr>
<tr>
<td>Protein</td>
<td>7.612 ± 0.141</td>
<td>6.370 ± 0.206</td>
<td>1.186 ± 0.028</td>
</tr>
<tr>
<td>Dry weight</td>
<td>9.097 ± 0.091</td>
<td>7.293 ± 0.130</td>
<td>1.263 ± 0.033</td>
</tr>
<tr>
<td>Protein</td>
<td>7.458 ± 0.109</td>
<td>5.694 ± 0.176</td>
<td>1.305 ± 0.036</td>
</tr>
<tr>
<td>Dry weight</td>
<td>9.173 ± 0.202</td>
<td>7.293 ± 0.130</td>
<td>1.263 ± 0.033</td>
</tr>
<tr>
<td>Protein</td>
<td>7.329 ± 0.098</td>
<td>5.694 ± 0.176</td>
<td>1.305 ± 0.036</td>
</tr>
</tbody>
</table>

*These ratios were calculated according to the following equation:

\[
\frac{\text{control}}{\text{X-ray (mean)}} = \frac{\bar{y}}{\bar{x}} = \frac{\bar{y}}{\bar{x}} \left(1 + \frac{\sigma_x^2}{\bar{x}^2} - \frac{r_{xy} \sigma_x \sigma_y}{\bar{xy}}\right)
\]

\[
\left(\text{standard error of the mean ratio}\right)^2 = \frac{1}{n} \left(\frac{\bar{y}}{\bar{x}}\right)^2 \sigma_y^2 + \frac{\sigma_x^2}{\bar{x}^2} - \frac{2r_{xy} \sigma_x \sigma_y}{\bar{xy}}
\]

REFERENCES


It seems important to determine whether certain indices of functional capacity would be useful in sorting out constitutional factors which presumably contribute to the variability in radiation effects. As an approach to this problem, we have attempted to evaluate a parameter of physical fitness in terms of acute lethality after whole body irradiation. Mice (CF #1 female, 20 g) were divided into groups of good, average, and poor on the basis of the time required for exhaustion while swimming. Ten per cent of 120 mice changed their classification from poor to good or vice versa during a second test. These animals were discarded from the experiment. Only those animals that did not change their classification in the two swimming trials separated by one week were retained in the good and poor groups, the remainder were classified as average. Mice were irradiated with 650 r, one week after the second test. There were no significant differences in the 30-day survival data among the three groups. It is possible that physical fitness may be correlated with sensitivity to other sequelae, for example, certain of the more chronic effects resulting from single or repeated low-dose irradiation.
Since low energy gamma rays are included among the radiations emitted from fission product clouds, it is of interest to determine the biological effectiveness of these rays. For this purpose, laboratory studies of mice and rabbits and of their phantoms were employed under conditions described below. Evidence in support of the mean body dose hypothesis(1,2,3,4) was used as a provisional guide in the specification of dosimetry measurements. Although the biological results have been reported at intervals(5,6,7) since the inception of these studies, the present summary is confined to a discussion of physical data.

The dosimetry problem was to obtain depth dose values within mice, rats and rabbits when exposed to various energies of X rays administered so as to simulate gamma radiation from an ambient cloud of fission products. Each particle comprising such a cloud would behave as a point source of radiation. This condition was approximated by lateral exposure about the antero-posterior axis in eight equally-spaced aspects. Peak dose in depth for the rabbit exposed from a single aspect was used as a guide in determining fore- and backscatter requirements with respect to both mouse and rat targets. The accompanying picture (Figure II-3) shows the general features thus established to place the beam of primaries in equilibrium with secondaries for the smaller target sizes, as would be the case under field conditions. Comparable spectral quality of the entrance dose at all energies (summarized in Table II-8) and target sizes were thus approximated. Exposure geometry of each test animal and its corresponding phantom was identical.

Representative weights for each species served as a guide in establishing phantom size, an equal number of animals of both sexes being used in all cases. Total body volumes were determined by displacement in water and corrections for both lung and fur bias were applied,(8) after which overall body length and posture within the exposure cage was found by a series of radiographs. Cross section area and shape was then computed at all points along the body axis for each species. It was found that an elliptical cross section (\( e = 1.39 \pm 0.01 \)) was most characteristic of the NLI rabbit, while circular cross sections best served to approximate both the Sprague-Dawley rat and the BA F1 mouse.

The classical proofs that the biological effects of radiation (at energies comparable to those employed in the study here reported) on *Drosophila melanogaster* eggs,(9) *Ascaris megalopehala* eggs,(10) or other very small biological targets, are independent of wavelength, would seem to have no validity in any effect dependent on the total energy absorption
in a large animal\(^{(2)}\). An ideal test of this provisional conclusion would involve a detailed measurement in depth for the animal exposed to various X-ray energies, each with a characteristic mean effective wavelength.

The relatively unwieldy size of a thimble ionization chamber compared with that of a mouse body led to a search for some means of measurement involving a detector of much smaller size. At that time, neither wavelength-independent emulsions\(^{(11)}\) nor cadmium sulfide scintillation crystals, since shown to be in linear correspondence with the Victoreen thimble ionization chamber from 80 to beyond 250 pkv,\(^{(12)}\) were available. Partial success was achieved with film dosimetry, largely through the efforts of A. S. Tracy. Using the Victoreen response as a standard of calibration, a technique of handling and processing which required closer than color film tolerances (using Ortho Thin Base Type 2 Film) was evolved so that optical density changes in the film were in linear correspondence with the thimble chamber readings both in air and in presdwood phantom depth. Though in close agreement at 80 pkv, film response departures from linearity with chamber response at energies beyond 100 pkv precluded the use of film data for the major portion of the range (upper limit at 250 pkv) in question. Thimble chamber data (Figure II-5) indicate that for targets the size of a mouse the axial dose is nearly constant (± 3%) over the entire range from 80 to 250 pkv. Film dosimetry was therefore unnecessary. No doubt the situation in depth would have been quite different for unilateral exposures without the presence of fore- and backscatter boards.\(^{(13)}\)

Discrepancies in surface and volume relations between animals and phantoms were kept to a minimum by the use of presdwood with a mass density of 1.00 ± 0.03 gm/cc, the organismal average for the species tested. Calculations based on data kindly furnished by various sources,\(^{(14,15,16)}\) here gratefully acknowledged, show the mean atomic number (Z) for presdwood is 3.98. Comparable data from Lea\(^{(17)}\) indicate a mean Z-value of 3.55 for wet tissue. Since ion density produced by X-ray bombardment increases with atomic number,\(^{(18)}\) these phantom measurements are therefore taken to represent energy absorption values slightly in excess of those actually present within test animals under comparable conditions of exposure.

The accompanying figures and tables describe the conditions of exposure and results therefrom. Figures II-2 and II-3 show the “ferris wheel” arrangement in relation to the X-ray machine head which could be rotated through a total arc of 180° in a vertical plane. Phantoms were reversed 180° about the antero-posterior axis following irradiation from four aspects in 45° increments of the vertical plane through the machine head, after which the other four aspects were employed. This same procedure was used for mouse exposures; concurrently with reversal in heading, the phantom mouse was relocated diametrically opposite its initial locus (aspects 1-4) on an isodose circle. Figures II-4 - II-6 summarize depth dose data thereby obtained and Tables II-7 and II-8 show energy absorption at various kilovolt values and their modes of generation respectively. In addition to the
TABLE II-7

Phantom Response to Ambient Low-energy X rays (10 r/min., air)

<table>
<thead>
<tr>
<th>Phantom mass (gm)</th>
<th>Energy (pkv)</th>
<th>Integral body dose (gm-r/min)</th>
<th>Mean tissue dose (r/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLI 3.0 x 10³</td>
<td>80</td>
<td>19.2 x 10³</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>23.0 x 10³</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>27.8 x 10³</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>27.9 x 10³</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>28.6 x 10³</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>28.6 x 10³</td>
<td>0.95</td>
</tr>
<tr>
<td>S-D 2.2 x 10²</td>
<td>80</td>
<td>1.68 x 10³</td>
<td>7.64</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>1.87 x 10³</td>
<td>8.50</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1.87 x 10³</td>
<td>8.50</td>
</tr>
<tr>
<td>Ba F₁ 22</td>
<td>80</td>
<td>1.96 x 10²</td>
<td>8.73</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>2.04 x 10²</td>
<td>9.27</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>2.04 x 10²</td>
<td>9.27</td>
</tr>
</tbody>
</table>

TABLE II-8

Conditions of Irradiation, G. E. Maxi mar X-ray Machine

<table>
<thead>
<tr>
<th>Peak kilovoltage</th>
<th>Filtration</th>
<th>Half value thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>air</td>
<td>2.8 mm Al</td>
</tr>
<tr>
<td>100</td>
<td>1.25 mm Al</td>
<td>4.5 mm Al</td>
</tr>
<tr>
<td>135</td>
<td>6.85 mm Al</td>
<td>4.2 mm Al (or 0.17 mm Cu)</td>
</tr>
<tr>
<td>150</td>
<td>0.25 mm Cu + 2.0 mm Al</td>
<td>0.2 mm Cu</td>
</tr>
<tr>
<td>200</td>
<td>1.25 mm Cu</td>
<td>1.8 mm Cu</td>
</tr>
<tr>
<td>250</td>
<td>3.25 mm Cu</td>
<td>3.3 mm Cu</td>
</tr>
</tbody>
</table>

Note: Distance from cathode to center of target = 76 cm.
Inherent filtration at 80 pkv = 3 mm Al. Air dose rate at centerline of each target = 10 ± 1 r/min.
See text.
Figure II-2 Rabbit exposure cage showing counterweights for lateral stability.

Figure II-3 Mouse exposure cages with scattering boards in place; essentially the same arrangement was employed for phantom rat dosimetry.
Figure II-4  Depth dose response within 3 kg rabbit phantom.

Figure II-5  Dose at the center of mass for mouse, rat, and rabbit phantoms.

Figure II-6  Average depth dose within mouse, rat, and rabbit phantoms.
conditions of Table II-8, exposures of mouse and rat phantoms also employed the presdwood fore- and backscatter boards mentioned earlier.

Under the conditions of ambient exposure as here employed, the following conclusions emerge: (1) irrespective of biological target size, there is no appreciable change of dose in depth from 135 to 250 pkv; (2) the dose in depth for a mouse target remains essentially constant on exposure to X rays with energies from 80 to 250 pkv; and (3) at energies of less than 135 pkv, ambient X-ray exposure for phantoms in the range of rabbit body mass is accompanied by a definite decrease of dose in depth.

ACKNOWLEDGEMENTS

In addition to those acknowledged in the text, the writer is particularly indebted to the following members of the Division: George A. Sacher for many helpful suggestions; A. S. Tracy for photographic dosimetry and the pictures in this article; all members of the X-ray Group, especially Jean L. Drallmeier; Juanita Lestina for the species radiographs; Philip F. Gustafson for the determination of half value layers; H. E. Kubitschek and Douglas A. Grahn for their many helpful discussions.

REFERENCES


7. Grahn, D., G. A. Sacher and H. Walton, Jr. 1954. Comparative biological effect of 80 kv and 250 kv X rays on the BA F_1 mouse. ANL-5332, 7-10


THE GAMMA RAY TOXICITY PROGRAM:
CALIBRATION AND EQUIPMENT

G. A. Sacher, D. Grahn, S. Lesher, and K. Hamilton

In the past quarter the calibration of the low-level gamma room was completed and the long-term program of investigation was begun.

The calibration procedure and most routine dosimetric procedures in the gamma facilities are based on the use of the Victoreen roentgen rate meter and Victoreen condenser chambers. Our secondary standard of the gamma ray roentgen is a 25 r condenser chamber with a Lucite cap 4 mm thick that was calibrated by the National Bureau of Standards. Cross-checks have also been made with the substandards maintained by other laboratories.

The Victoreen rate meter has shown itself to be a rugged and stable instrument. Some modifications were necessary to attain maximum precision of measurement. Readings were made by potentiometric measurement instead of meter deflections. The expansion of scale and elimination of non-linearity permit a reading accuracy of about 1/10% of full scale. Calibration factors between the 4 probes and 4 input levels could then be determined with adequate accuracy. The reproducibility of measurement is within 1% over a period of several months and over-all consistency between probes and input levels is believed to be within 2%.

A plot of the dose-rate curves found on a vertical plane through the axis of the source is given in Figure II-7. The isodose surfaces are radially symmetrical within 1% at all positions tested. The source consists of a cobalt rod 12 in. long x 1/4 in. diameter. Its strength at present is about 7.8 curies. For this calibration the source was in its standard position with its midpoint 47.3 in. above floor level. A more detailed presentation of procedures and results will be given at a later time.

The principal use of the low level gamma rooms is for duration-of-life irradiation of experimental animals. Containers must therefore be devised that are satisfactory with regard both to husbandry and exposure geometry. The mouse container developed after a lengthy period of consultation and experiment is shown in Figure II-8. The containers are fabricated from a transparent Bakelite. They are inexpensive and durable. The cylindrical shape eliminates the possibility of position preferences that would increase the error variance of the radiation doses.

The containers may be used for one to three mice. At an early stage in planning, complete isolation of mice was considered, but the final decision was to use cage groups of three mice. Caging in groups of three is less costly, and also it gives opportunity for investigating intra-cage factors such as disease transmission.
Figure II-7  Intensities measured in low-level gamma room as a function of horizontal distance from source; for 7 distances from floor corresponding to the shelf heights used for chronic exposure. Plots of intensity vs. distance between source and probe centers approximate very closely to the inverse square law for the range of distances covered in this figure.
Figure II-8 Cage used for irradiation of mice for the duration of life.

Figure II-9 View of low-level gamma room from doorway. Features shown include: source drive mechanism, guide tube and lead shield around lower 3 feet of guide tube; zinc bromide window at right rear; dosimeter probe and probe positioner at left; aluminum racks with cages in position. The source is stored below floor level in storage shield. Ceiling and upper wall corners covered with acoustic tile to prevent annoying echoes.
The containers are positioned within the room on aluminum racks. The arrangement of racks within the gamma room should be such that about the same number of animals per year can be put through the room at daily dosages ranging from 220 down to 6 r per day. The best arrangement to satisfy this requirement consists of a spiral. Figure II-9 shows the room shortly after the definitive experiment began, with 180 mice in 60 cages to receive six different daily dosages ranging from 220 down to 24 r. Since the photograph was taken mice have been entering at a rate of about 400 per month. The rate will increase somewhat in the near future.

Exposures are given by raising the source for a fixed time each day. This time is 12 hours at present. The exposure time is increased in 5 minute steps at predetermined intervals to compensate for radioactive decay.
RECOVERY AND REGENERATION OF SPLEENS AFTER SINGLE AND DIVIDED DOSES OF X-IRRADIATION

Agnes N. Stroud

Previous reports\(^1,^2\) have considered the weight changes occurring in the spleens of 7-10 week CF #1 female mice irradiated at weekly intervals. One week after the first exposure a marked, dose-dependent, weight loss is observed. This is followed by a slight recovery after the second exposure and a marked return to and beyond control weight values following the third exposure. After this compensatory increase, weights decrease and begin to recover again after the sixth and last exposure (Figure II-10).

It was of interest to compare these compensatory effects with those occurring in the spleen and other organs after single doses of irradiation. Table II-9 shows the effects of several, single, total body X-ray doses, including 800 r preceded by injection of 1 cc mouse serum (designated as M.S. + 800 r), on spleen, thymus, and body weight. Figure II-11 represents the spleen weight data graphically. Observations of spleens were not made 4 and 7 days after 400 r, but Carter et al.,\(^3\) report a decrease of 60% from control weights at 5 days post-irradiation. The decrease in spleen weight 4 days after administering the higher doses is about 80%. At 800 r, a dose for which the maximum survival is 14 days, there is no recovery of the spleen. The spleens of animals surviving 550 r or pretreatment with mouse serum + 800 r (LD\(_{50}\) = 30 days) begin to show recovery and increase in weight by one week post-irradiation. By two weeks they are approaching control values, and in the case of the 550 r spleens, have reached a maximum value at 17 days and begin to fall off after that time. Spleens of the 400 and pretreated 800 r group reach their peak at 21 days.

It is noted that the time of maximum recovery is three weeks after either a single, large dose or after the first of a series of weekly, divided, doses. In the latter case, recovery occurs during the course of radiation treatments. It is seen that recovery is more rapid after a single radiation dose. With periodic doses there is a certain amount of injury with each additional dose, which is more apparent in the case of the higher dosages, but the spleen at the same time recovers, to some extent, from one week’s exposure to the next.

Between 12 and 15 days after irradiation, spleens from the single-dose exposures had many nodules on the cortex. From Figure II-11, spleen weights for 400, 550, and 800 r (pretreated with mouse serum) approach control values at 15 days. These nodules are not seen at 17 days or thereafter when the spleen weights have gone beyond control values. Histological examination reveals that these are cellular areas with high mitotic activity and appear to be reticular\(^4\) cells. Mitosis is suppressed, or almost
Figure II-10 Effect of 67, 133 and 200 r/week on spleen weight (C571O mice) (Arrows indicate times of irradiation).

Figure II-11 Spleen weights post-irradiation.
TABLE II-9

Body weight, spleen, and thymus weight changes after a single dose of X-irradiation (body wt. in grams, organ weights in mg)

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>DOSE (r)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>7</td>
<td>12</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>Spleen</td>
<td>0</td>
<td>107.6</td>
<td>90.0</td>
<td>92.2</td>
<td>---</td>
<td>92.7</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>---</td>
<td>---</td>
<td>77.8*</td>
<td>---</td>
<td>161.3</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td>22.6</td>
<td>27.0</td>
<td>36.1</td>
<td>198.7</td>
<td>181.9</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>22.4</td>
<td>21.4</td>
<td>17.2</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>M.S.+800**</td>
<td>16.0</td>
<td>19.3</td>
<td>47.6</td>
<td>---</td>
<td>164.9</td>
</tr>
<tr>
<td>Thymus</td>
<td>0</td>
<td>68.5</td>
<td>52.0</td>
<td>68.3</td>
<td>---</td>
<td>54.8</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>56.4</td>
<td>---</td>
<td>63.2*</td>
<td>---</td>
<td>46.6</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td>10.5</td>
<td>16.0</td>
<td>25.3</td>
<td>25.2</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>10.7</td>
<td>5.3</td>
<td>8.2</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>M.S.+800**</td>
<td>10.6</td>
<td>4.7</td>
<td>40.9</td>
<td>---</td>
<td>28.7</td>
</tr>
<tr>
<td>Body Wt.</td>
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<td>20.0</td>
<td>20.4</td>
<td>---</td>
<td>22.6</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>19.6</td>
<td>---</td>
<td>23.1</td>
<td>---</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td>16.0</td>
<td>19.0</td>
<td>19.1</td>
<td>---</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>17.0</td>
<td>16.1</td>
<td>12.9</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>M.S.+800**</td>
<td>15.7</td>
<td>16.4</td>
<td>18.7</td>
<td>---</td>
<td>19.4</td>
</tr>
</tbody>
</table>

*14 day figures
**= Mouse serum (1 cc I.V. pre-irradiation)
completely absent, at 4 days post-irradiation. Between 12 and 17 days one finds the mitotic activity to be at a maximum, and by 21 days it has decreased almost to control values. Although nodules are not present after 17 days, these regenerating areas are found near the periphery of the spleen if longitudinal sections are made. After the 3rd and the 6th in the series of weekly divided doses these nodules are not observed but the spleens have a corrugated appearance, which corresponds to the foci of regeneration seen 17 days after a large single dose of radiation.

Having made these observations on compensatory regeneration of cells in the post-irradiated spleens, and with the knowledge that spleen homogenates from 2-week-old mice give a certain degree of protection to mice after an LD₁₀₀ dose of X-irradiation, it was of interest to test these spleens for protective activity.

Three groups of mice were exposed to whole body X-irradiation. One group received 550 r, another 400 r, and the third 400 r with the spleens shielded during irradiation. Two weeks after irradiation, and at the time when the cellular nodules were evident and weights had returned to control values, the spleens were removed under sterile conditions and homogenized in a glass homogenizer. Thirty to fifty mg of this brei were injected intraperitoneally into mice which had received 725 r. The best results were observed in the groups which received either 50 mg of shielded-spleen homogenate (400 r) or 40 mg of non-shielded spleen. The 21-day survivals were 15% and 10%, respectively. There were no survivors with 550 r spleen homogenate. Spleens taken 25 days after irradiation, at both doses, were tested and found to have no activity. Since there appears to be some evidence of activity in the recovering spleens, experiments are in progress by H. Ducoff and the author to find the time at which the splenic cells are most effective.

It is noted in Table II-9 that the thymus weight decreases sharply 4 days after a single dose of 550 and 800 r. Recovery of the organ is more dramatic in mice treated with mouse serum before receiving 800 r (apparent dose, 550 r) than with the 550 r group. By 12 days the thymus of the pretreated group is back to control values, with a slight decrease at 21 days. The 550 r group never reach control values; at 12 and 17 days their weights are found to be 35% of the controls. Histological examination of the recovered thymus reveals no reticular cell regeneration as seen in the spleen. Mitotic activity is high but mitosis is primarily confined to the thymus cells.
REFERENCES


PILOT STUDY ON IRRADIATION OF BIOLOGICAL MATERIALS AND CARCINOGENICITY

Austin M. Brues, Katherine A. Hamilton and Howard Walton, Jr.

It has been observed that pure cholesterol, irradiated in the reactor to about $10^9$ rep, fails to acquire carcinogenic activity (ANL-4713, p. 80). Since chemical reactions in the presence of water and other materials will necessarily be considerably different from those in the dry, pure state, and in view of current interest in sterilization of foods by high level irradiation, it was thought desirable to extend these observations using biological material containing cholesterol and many other compounds which might be converted to carcinogens.

Two fresh, infertile egg yolks were mixed and divided into two portions. One of these was irradiated in the central thimble of the CP-3 reactor while the other was maintained at a temperature equal to that of the one being irradiated. The total amount of fast neutron irradiation is estimated to be on the order of $10^4$ rep. Aliquots were separated for determination of induced activity, chemical analysis, and bioassay on mice.

After 5 days the only measurable activity appeared to be that of $^{32}$P. Repeated extraction was made with acetone and benzene; the resulting solutions were dried and dissolved in petroleum ether (2 ml per gram of original yolk). The irradiated material was somewhat lighter in color than the control sample, and spectrophotometry showed a reduction in the absorption peaks at 431, 455, and 481 μμ and a small increase in the peaks at 335 and 365 μμ. No new peaks were found. Chromatography was done on these solutions, using an alumina column and ethyl ether as eluant. Eighteen fractions were collected from each sample and examined for visible fluorescence under ultraviolet light; no noticeable differences in fluorescence were seen in comparable eluates of the two samples. In particular, the first sample (which carries out almost all of the methylcholanthrene from an egg yolk extract with the carcinogen added) showed no fluorescence, whether derived from the control or the irradiated yolk.

Bioassay was done by injecting the irradiated yolk subcutaneously in the mid-backs of young female CF #1 mice. Four mice received 0.5 ml, an equal number, 0.2 ml, and 15 mice were injected with 0.1 ml. Ten mice had previously received 0.5 ml, and 10 mice, 0.25 ml, of unirradiated yolk. The experimental injections were made 4 months after irradiation, when the $^{32}$P of the largest injection doses was less than $10^{-3}$ μμ. Two of the mice in the lowest dosage group died without tumor between 12 and 13 months after injection; all of the other mice are still living at 13-1/2 months. One of the four injected with 0.5 ml of irradiated yolk has a small papilloma near the site of injection; no significance needs to be attached to this isolated
observation, since pure cholesterol is known to be a mild carcinogen,\(^{(1)}\) and since many other known constituents of egg yolk have apparently not been tested for carcinogenic activity.

More extensive experiments along similar lines are now being planned.

REFERENCE

RADIO-RUTHENIUM TOXICITY STUDIES

H. Walton, Jr. and A. M. Brues

Previous biological studies on radio-ruthenium have dealt with distribution of tracer amounts in rats after intramuscular and intraperitoneal injection and inhalation of RuO₄.(¹,²) The present report deals with acutely lethal effects in mice which received fission ruthenium intravenously.

In selecting dose levels, cognizance was taken of the LD₅₀-30 days for mice of Sr⁸⁹, previously established at 8 μc/gm body weight,(³) and the greater maximum beta ray energy of Rh¹⁰⁶ (3.5 Mev) which is the predominant energy in the decay pattern of Ru¹⁰⁶. It was anticipated that the LD₅₀-30 days would lie between 1 and 5 μc/gm, most likely between 3 and 4, based on comparison with Sr⁸⁹. Dosages of 8.94, 4.96, 3.21, 1.42, and 0.57 μc/gm were actually employed.

On receipt, the radio-ruthenium chloride had an activity of 2.8 mc/ml and a concentration of 0.20 mg/ml. Absorption curves for beta rays indicated that the mix consisted of 90% ruthenium-106 and 10% ruthenium-103. The presence of several valence states was confirmed by electrochromatography. The injection solutions as made up varied in pH and were approximately isotonic. Mice were injected via the caudal vein in amounts from 0.1 to 0.5 ml, depending on the dose. Calibration aliquots of each dose level which served as standards during subsequent activity measurements were obtained at the beginning and at the end of the period during which animals were injected. Forty randomized 15-week-old animals were employed; ten at each of the three intermediate levels, and five at either extreme of the range investigated.

A pulse height analyzer in conjunction with a NaI-Tl activated scintillation crystal was used for all activity measurements. The crystal was aluminum-shielded to reduce beta ray intensities, and absorption curves in water under these conditions indicated that gamma ray counting beyond 0.5 Mev for the doses employed was mass-independent. It was therefore possible to do whole body counting in vivo and wet tissue counting at death. The total number of counts for each activity measurement exceeded 10⁴ and therefore the statistical error of counting was taken to be less than one per cent.

The data so far obtained are summarized in the figures and table. In Fig. II-12 we see the relation between dose and survival: the 30-day LD₅₀ appears to be about 3.5 μc/gm, as anticipated. Figure II-13 shows the total retention during the first few days, indicating that about half of the dose is eliminated by the twelfth day. This is comparable with the 20-day biological "half-time" indicated by Scott and Fisher.(¹) The rapid early excretion observed by these workers (about 25% in the first day and 50% by the end of the
Figure II-12  Relation of time of death to radio-ruthenium dosage.

Figure II-13  Retention of ruthenium dosages from 0.57 to 8.9 $\mu$C/gm body weight.

Figure II-14  Whole body concentrations at acutely lethal doses ($\text{Ru}^{106}$).
fourth day} after intraperitoneal administration has not been observed by us, perhaps owing to the route of administration. It also appears that the retention is unaffected by dose within the range studied. In fact, when the retention is corrected for body weight changes, as shown in Fig. II-14, it is seen that the total body specific activity rises in the second week for those animals at the highest dose level. Since starvation has no effect on the elimination rate of this radioelement (Fig. II-13), the rise in specific activity must be due to a loss in body weight.

The accompanying table summarizes terminal activity distributions for mice in the 30-day median lethal dose region and indicates (for acute cases, at least) that the material was handled in colloidal form. Departures from previously published values in rats are apparent and it is noted that the liver and spleen (and, eliminating the inhalation experiment, lung) show much higher values in our experiments. It is not known whether this is due to difference in species, route of administration, or amount administered, but the latter two differences might be expected to alter the colloidal state of the element in the blood stream and thus influence the tissue distribution. This may also be related to the reduced rate of elimination in our experiment.

Radiography of fatalities from the 1.4 μc/gm level (up to 6 months after injection) shows increased density of the long bones resembling the changes characteristically seen in mice treated with carcinogenic doses of bone-seeking radioelements. Mice are now being observed at longer periods after administration of lower dosages and the results will be reported in the future.

ACKNOWLEDGEMENTS

We are indebted to the following members of the Division for assistance in this study: Walter E. Kisieleski for preparation of the aliquots and for beta ray absorption data, T. Richard Sato for electrochromatographic analysis of the sample, Philip F. Gustafson for gamma ray spectra and calibration of the pulse height analyzer, Miriam P. Finkel for the benefit of her experience, A. N. Stroud for inoculating the animals, and Juanita Lestina for the radiographs.
### TABLE II-10

Organ Distribution of Ruthenium at Time of Death

<table>
<thead>
<tr>
<th>Organ or Tissue (CF#1Q)</th>
<th>Per Cent of Total Activity Present</th>
<th>LD₅₀-16 days Group</th>
<th>LD₅₀-36 days Group</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>26.5</td>
<td>25.0</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>9.3</td>
<td>6.6</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>3.3</td>
<td>3.1</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>3.1</td>
<td>2.2</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.4</td>
<td>2.3</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>2.1</td>
<td>1.8</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>1.1</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Salivary glands</td>
<td>0.6</td>
<td>1.0</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Remainder</td>
<td>51.2</td>
<td>56.6</td>
<td>53.9</td>
<td></td>
</tr>
</tbody>
</table>

Note: Stomach and intestine values include contents.

### REFERENCES


TOXICITY OF Sr\textsuperscript{89} IN MICE. MALIGNANT BONE TUMORS

M. P. Finkel, H. Lisco and A. M. Brues

The purpose, design, and conduct of the long-term radiostrontium study in mice, as well as information concerning survival and tumors among the control animals, have been reported previously (ANL-4840, -5247, and -5288). The present communication is restricted to an analysis of the malignant bone tumor data.

Many skeletal neoplasms were observed before the animals showed other evidence of disease, some were noted first at autopsy, and still others were detected during examination of the terminal roentgenograms. All but a few of the lesions were examined histologically; these were diagnosed from roentgenographic appearance alone because the specimen was lost. Of the 427 malignant tumors originating in bone that were detected among the 3083 animals in the experiment, 92% were classified as osteogenic sarcomas, 3.7% as fibrosarcomas, 0.5% as undifferentiated sarcomas, and 3.7% as hemangioendotheliomas.

Although the CF#1 female mice comprised only one-third of the total population, they possessed 54% of the tumors (Table II-11), and although the ABC mice were equally divided between male and female, the two sexes accounted for 18% and 28% of the tumors, respectively. The strain difference is exemplified by the fact that the CF#1 female mice had twice as many tumors as the ABC female mice and the sex difference by the fact that the ABC female mice had 1.5 times as many as the ABC male mice. The majority of the neoplasms occurred in the long bones; 48.6% of those in the CF#1 mice and 58.4% of those in the ABC mice were on the right side. Strain and sex differences were noted in the proportion of tumors that appeared in the spine and in the skull. The CF#1 female mice had 7 times as many neoplasms in the spine as in the skull whereas the ratio in the ABC female was 3:1 and in the ABC males 1:1. In spite of the variations in tumor incidence and location among the three kinds of mice, their responses to radiostrontium in terms of the induction of bone malignancies were comparable. Therefore, it seemed desirable to consider the three groups as a single population in the analysis of the bone tumor data since each contributed approximately equal numbers of animals to the specific dosage groups and since greater statistical reliability obtains with increased population size.

Incidence: The cumulative incidence of animals with malignant bone tumors after a single injection of strontium 89 is illustrated in Figure II-15. There is a positive correlation between dose and the final proportion of animals with tumors up to 2.5 \(\mu\text{c/gm}\) but this relationship is reversed at higher levels. A less pronounced damping effect is indicated at the highest monthly repeated
Although there is unequivocal evidence that a single dose of 0.2 \( \mu \text{c/gm} \) and monthly repeated doses of 0.1 \( \mu \text{c/gm} \) induced bone malignancies, the data for the lower doses are only suggestive.

**TABLE II-11**

Location of Malignant Bone Tumors

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of tumors</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CF#1♂</td>
<td>ABC♂</td>
</tr>
<tr>
<td>Long bones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>femur</td>
<td>70</td>
<td>49</td>
</tr>
<tr>
<td>tibia</td>
<td>61</td>
<td>22</td>
</tr>
<tr>
<td>humerus</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>138</td>
<td>77</td>
</tr>
<tr>
<td>Spine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cervical</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>thoracic</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>lumbar</td>
<td>38</td>
<td>13</td>
</tr>
<tr>
<td>sacral</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>caudal</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>26</td>
</tr>
<tr>
<td>Skull</td>
<td></td>
<td></td>
</tr>
<tr>
<td>minus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mandible</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>mandible</td>
<td>6</td>
<td>3</td>
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<tr>
<td>Total</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Other</td>
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<td></td>
</tr>
<tr>
<td>pelvis</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>clavicle</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>rib</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>patella</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>231</td>
<td>119</td>
</tr>
<tr>
<td>TOTAL POPULATION</td>
<td>1026</td>
<td>1031</td>
</tr>
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</table>
Figure II-15 Cumulative incidence of animals dying with malignant bone tumors after a single injection of radiostrontium. Dosage = μc/gm.

Figure II-16 Cumulative incidence of animals dying with malignant bone tumors after the first of monthly injections of radiostrontium. Dosage = μc/gm/month.
Comparisons between treated and control animals and between singly-injected and monthly-injected animals would be facilitated if the behavior of each dosage group could be described by a single value instead of by a series of values. The final cumulative incidence might be such a statistic except that it is distorted at high levels by mortality from acute irradiation disease and at all levels by death from incidental diseases that deplete the population before the animals have lived long enough to develop malignant bone tumors. These difficulties can be obviated by excluding from the calculations those animals that died too early to have tumors. Such a correction requires the positive identification of a latent period.

Latent period: Malignant bone tumors were found in 191 of the mice, each of which is represented in Figure II-17. No tumor-bearing animal died before the 180th day of the experiment (at which time the mice were approximately 250 days old), but once this interval had passed, many tumor deaths occurred. Although death with a tumor at 180 days necessarily implies that the malignancy was present earlier, the data do not permit reliable estimates of how much earlier this might have been. The clinical records and histological appearances of the cases indicate that some were very rapidly growing tumors and some progressed slowly. Nor do these data tell us what subtle biological change preceded the neoplastic process, how many cells were involved in the primary change, and how much time elapsed between this event and the appearance of tissue that could be called neoplastic. In spite of these deficiencies the existence of a latent period cannot be denied.

Is the length of the latent period dependent upon the size of the dose? If, in Figure II-17, attention is focussed on the time of death of the first tumor-bearing animal in each dosage group, it is seen that the interval increases from 2.5 to 1.0 to 0.5 \( \mu \text{C/gm} \) and from 0.5 to 0.2 to 0.1 \( \mu \text{C/gm/month} \). However, the time was not decreased at higher dosages and all evidences of a trend were lost among the lower dosages and controls. A minimum time requirement can be postulated to explain the results at the higher levels but no ready explanation consistent with dose-latent period dependence presents itself for the lack of trend among the low level and control groups. When attention is focussed on the average survival time of the tumor-bearing animals, 5 of the 15 dosage groups (0.2 \( \mu \text{C/gm} \), 0.05, 0.02, and 0.01 \( \mu \text{C/gm/month} \), and controls) do not fit the hypothesis that the length of the latent period is a function of the size of the dose. The impression of a probable relationship is due to the facts that (1) survival time decreases with increasing dose and (2) the incidence of malignant bone tumors increases with increasing dose. The former results in a sharp annihilation of the population at high levels, which is reflected in a shortened average time to death of the tumor-bearing animals. The latter results in the appearance of many tumors at high levels as soon as the latent period has passed because the probability of a tumor arising is so high; at the lower levels, where the incidence is low, the probability of such an event occurring as soon as the latent period is over
Figure II-17 Interval between initiation of the experiment and death with a malignant bone tumor.
is also low. This simple explanation accounts for those cases that do not fit the hypothesis that the length of the latent period is a function of the size of the dose, e.g., the early death of the animal that received 0.01 µc/gm/month.

Since these data support the views that a latent period for the induction of skeletal malignancies by radiostrontium in mice does exist and that the duration of this period is independent of the size of the dose, and since 180 days from the initiation of the experiment to death with a tumor adequately describes the observed situation, further statistical evaluations of the bone tumor data were based on the population that survived to 180 days after the beginning of the experiment.

Multiple tumors: The 191 tumor-bearing mice possessed 427 tumors; the number of tumors per animal ranged from one to fourteen. A decision had to be made as to whether the unit datum for further statistical procedures should be the tumor or the tumor-bearing mouse. If the tumors were distributed at random among the similarly treated animals, the number of tumors would be more significant than the number of animals with tumors; conversely, if the tumors were not randomly distributed, it would have to be concluded that there were tumor-prone and tumor-resistant animals or that multiple tumors were actually cases of metastatic lesions, and, therefore, the tumor-bearing mouse was the only valid unit. These data are best suited to be tested for the random occurrence of independent events by the Poisson method. For each dosage group in which there were animals with more than one tumor the Poisson distribution of tumors was calculated, and the actual distribution was compared with the theoretical distribution by the chi square test for goodness of fit. Ten of the 191 animals had improbably large numbers of tumors for their respective dosage groups; this was reduced to six when corrections were made for survival time. Four of these six animals had presented difficulties in the determination of tumor number because, although there was histological evidence of multiple primary tumor sites in the spine, these might have been resolved into a single tumor had serial sections been available. In spite of these exceptions, the great weight of evidence favors the hypothesis that the tumors are distributed at random among the animals exposed to similar risk. Therefore, the tumor rather than the tumor-bearing mouse is the more valid statistical unit.

Tumor expectancy is defined as the probability that an animal will possess a tumor at the time it dies, and it is calculated at successive intervals by dividing the number of tumors that are still to appear among the surviving population by the number of animals still alive. In Figure II-18 the average tumor expectancy from 180 days after the beginning of the experiment until less than four survivors remained is plotted separately for those animals that received single and monthly injections. The shape of the single-dose curve suggests that the skeletal system was so severely damaged at the higher levels that its capacity to produce neoplasms was diminished. Extrapolation to lower levels and to the control value of
Figure II-18 Expectancy of malignant bone tumors at death after various dosages of radiostrontium among animals that survived 180 days or more.

Figure II-19 Relative biological effectiveness of monthly repeated versus single doses of radiostrontium in producing malignant bone tumors.
0.002 tumors per animal permits the estimation of that level of strontium 89 in mice that has no effect on the induction of malignant bone tumors; this "indifference" dose lies between 0.001 and 0.005 μC/gm.

The two tumor expectancy curves can be compared most readily by converting to unity the curve that describes the response to a single injection. This has been done in Figure II-19, where it can be seen that multiple doses are much more effective than single doses in producing large numbers of tumors but that this difference in effect diminishes as the number of tumors decreases and, hence, as the size of the dose decreases. The damaging effect of high doses is strikingly demonstrated in the marked increase in effectiveness of repeated injections over single injections in the range where 70 to 90 tumors per 100 animals are produced.

Monthly repeated injections with a relatively short-lived isotope approach the situation that might obtain with a long-lived isotope of the same element. In addition, comparison of the effects of a dose given once with those of the same dose given every month as long as the animals live permits evaluation of a single insult versus repeated insults. The preceding examination of the data was made with such objectives in mind. However, it seemed possible that the data might also furnish some information on the effects of dose fractionation. Since the experiment was not designed for such a purpose, analyses are complicated by the facts that injections continued month after month, and that, after the initial injection, only 80% of the dose level was administered monthly. Nevertheless, some interesting results have been obtained. The animals that received more than one injection were regrouped according to the total dose that they had received 180 days before death. Classification on the basis of the dose present 180 days before death was necessary since, if a latent period exists after a single injection, it must also exist when the dose is fractionated. It is evident that the incidence of tumors among animals that received 1 μC/gm in one injection could not be validly compared with the incidence among animals that received 1 μC/gm at the rate of 0.5 to 1.0 μC/gm/month (initial injection of 0.2 μC/gm followed by monthly injections of 0.16 μC/gm) since the latter would have died between the 120th and 150th day of the experiment, which interval precedes the termination of the latent period.

The data are graphically presented in Figure II-20, where the following relations emerge: (1) if the total dose exceeded 1.0 μC/gm, more tumors appeared when that dose was given at the rate of 0.5 to 1.0 μC/gm/month than when it was given all at once; (2) when the dose rate was 0.1 to 0.2 μC/gm/month, fewer tumors resulted than when the entire dose was given at one time. These data have been converted to a more easily understood form in Figure II-21 by expressing the single-dose curve as unity. This shows, for example, that a total dose of 2.5 μC/gm given at the rate of 1.0 to 0.5 μC/gm/month is more than twice as effective as a single injection in producing bone malignancies but that when it is given at the rate
Figure II-20  Effect of dose rate upon the incidence of malignant bone tumors among 180-day survivors. The numbers of tumors and animals in each group are given in parenthesis to indicate the reliability of each point. There were three tumors among the 844 control animals, or 0.004 tumors per animal.
of 0.2 μc/gm/month it is only half as effective as a single injection. This analysis can be carried one step further, as has been done in Figure II-22, where it can be seen that the relative biological effectiveness in producing malignant bone tumors increases with increasing fractionation at high levels but decreases with increasing fractionation at low levels. The dividing point is relatively constant at approximately 0.25 μc/gm up to 5 fractionations. This indicates that when the dose is given at the rate of 0.25 μc/gm/month the biological effects of the individual injections are additive, and the final result is equal to that which would obtain if the dose had been given at one time. At lower doses the effects of the individual fractions do not add up to one. This suggests that some degree of recovery occurred between injections. Except for the similarity in response to 0.45 μc/gm given twice and 0.9 μc/gm given once, at doses higher than 0.25 μc/gm the effects of fractionated doses far exceed the effects of the same total dose given at one time. These results again demonstrate the increasing damage produced in the skeleton by increasing doses above 1.0 μc/gm. The optimum carcinogenic dose lies well below the dose that results in widespread cell death, a fact which is best illustrated by the relative biological effectiveness of 6:1 for 4.2 μc/gm given in 5 fractions as compared to 4.2 μc/gm given in a single injection.

Summary: Four hundred twenty-seven malignant bone tumors were found among 191 of the 3083 mice that comprise the strontium 89 toxicity experiment. The animals were equally divided among CF#1 females, ABC females, and ABC males. One-third received a single injection, one-third received monthly injections, and one-third served as controls. The following observations were made.

1. Ninety-two per cent of the malignant bone tumors were osteogenic sarcomas, 3.7% were fibrosarcomas, 0.5% were undifferentiated sarcomas, and 3.7% were hemangioendotheliomas.

2. The CF#1 mice had twice as many tumors as the ABC mice, and the ABC females had 1.5 times as many as the ABC males. In each group the majority of the tumors occurred in the long bones, but the relative proportions in the spine and skull varied from 7:1 in the CF#1 females to 3:1 in the ABC females to 1:1 in the ABC males.

3. The length of the latent period for the induction of malignant bone tumors was independent of the size of the dose. For purposes of this study the latent period is adequately defined as 180 days from the initiation of the experiment to death with a tumor.

4. With few exceptions malignant bone tumors were distributed at random among the animals exposed to similar risk so that cases of individuals with multiple tumors were common at optimum carcinogenic doses.
Figure II-21 Relative effectiveness of dose rate in inducing malignant bone tumors with radiostrontium.

Figure II-22 Relative effectiveness of number of fractionated doses in inducing malignant bone tumors. Since 100% of the dose level was injected initially and 80% of the dose level thereafter, the average fractionated dose is plotted.
5. The probability of developing a malignant bone tumor increases with increasing dose until that point where widespread tissue death reduces the capacity of the skeleton to produce neoplasms. The "indifference" dose is estimated to lie between 1.0 and 5.0 μC/kg.

6. Monthly repeated doses are more effective than single doses in producing bone tumors. The relative biological effectiveness increases as the response increases.

7. A total dose of 0.25 μC/gm results in the same number of bone tumors whether it is given in 1, 2, 3, 4, or 5 fractions. Smaller doses result in fewer tumors as the number of fractions increases and as the size of the dose decreases. Larger doses result in greater numbers of tumors as the number of fractions increases and as the size of the dose increases.

8. The most effective carcinogenic dose of strontium 89 for the mouse skeleton that was used in this experiment was 4.2 μC/gm given as an initial injection of 1.0 μC/gm followed by 4 injections of 0.8 μC/gm/month.
Acute survival curves for CF #1 female mice, after 90-minute exposures to either fast neutrons or Co\textsuperscript{60} gamma rays, have already been reported\textsuperscript{(1)}. During these experiments 2102 mice were exposed in the gamma-neutron radiation chamber\textsuperscript{(2)} and 1027 died within the 30-day period following such exposures. The data presented in this report indicate the daily probability of death for these mice during the 30-day post-irradiation period.

The animals exposed to each of the two ionizing radiations were divided into three dosage groups based on comparative mortality during the acute period. Thus, the highest group, whether neutron- or gamma-irradiated, showed approximately 80\% mortality, the mid-third roughly 50\%, and the lowest group approximately 20\% mortality (Table II-12).

Death checks were made twice daily after irradiation. The day of exposure was day zero, and all deaths thereafter were recorded on a noon-to-noon basis. The probability of death during each 24-hour period was calculated by dividing the number of mice that died during that day by the total number of exposed animals alive at the beginning of that day. This daily probability of death for each of the three groups is illustrated graphically in Figure II-23A for the neutron-exposed mice and in Figure II-23B for the gamma-irradiated mice.

The data presented indicate clearly that fast neutron-irradiated mice showed an earlier mortality pattern than the gamma-irradiated mice, and that this difference in mortality was apparent over the entire lethal range. Comparative mean survival times of the 30-day decedents are listed in the last column of Table II-12.
TABLE II-12

Acute Mortality of CF #1 Female Mice Following Single, 90-Minute Exposure to Ionizing Radiations

<table>
<thead>
<tr>
<th>Dose range</th>
<th># mice exposed</th>
<th># mice dead in 30 days</th>
<th>30-day mortality (%)</th>
<th>Mean survival time of 30-day decedents (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Fast neutrons:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>224-269 rep</td>
<td>328</td>
<td>269</td>
<td>80</td>
<td>7.4</td>
</tr>
<tr>
<td>207 rep</td>
<td>229</td>
<td>102</td>
<td>45</td>
<td>9.3</td>
</tr>
<tr>
<td>173-195 rep</td>
<td>391</td>
<td>76</td>
<td>19</td>
<td>10.4</td>
</tr>
<tr>
<td>Totals:</td>
<td>948</td>
<td>447</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Co60 gamma rays:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>997-1091 r</td>
<td>406</td>
<td>338</td>
<td>83</td>
<td>13.6</td>
</tr>
<tr>
<td>909-974 r</td>
<td>252</td>
<td>131</td>
<td>52</td>
<td>14.9</td>
</tr>
<tr>
<td>751-874 r</td>
<td>496</td>
<td>111</td>
<td>22</td>
<td>14.8</td>
</tr>
<tr>
<td>Totals:</td>
<td>1154</td>
<td>580</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES


Figure II-23  The daily probability of death of CF#1 female mice following single, 90-minute exposure to fast neutrons (A) and following similar Co$^{60}$ $\gamma$-irradiation (B).
THERMAL NEUTRON CONTRIBUTION TO A FAST NEUTRON EXPOSURE

H. Walton, Jr.

Extensive experiments in the past have dealt with the toxicity of fast neutrons. In recent experiments conducted in this laboratory by Vogel, Clark, and colleagues, a special radiation chamber has been used in which fission neutrons are obtained by placing a uranium converter in the thermal column of a reactor and using appropriate shielding to reduce gamma radiation and thermal neutrons.\(^{(1)}\) It has been thought desirable to make a direct estimate of the thermal neutron flux which might be present in the body of an exposed mouse using activation measurements, which are highly sensitive.\(^{(2,3)}\) In a preliminary measurement, the blood and livers of two mice treated at the LD\(_{95}\)-30 days, which is in the vicinity of 240 rep,\(^{(4)}\) were dried and placed under an end-window counter, and did not contain sufficient amounts of activation products to measure in this way. This indicated that a very small proportion of the neutrons had become thermal-ized, and accordingly it was necessary to employ a more sensitive technique.

Following a suggestion by H. E. Kubitschek, use was made of a copper foil, taking advantage of the nuclear reaction Cu\(^{63}(n,\gamma)Cu^{64}\) which is assumed to have a cross section of 4.3 barns for thermal neutrons.\(^{(5)}\) In one exposure the bare foil was used (weighing 221 mg) and in a second exposure it was surrounded by a cadmium envelope 0.4 mm in thickness to absorb the thermal neutrons. Neglecting the proportion of thermal neutrons transmitted by the cadmium (estimated as less than 1% of those ambient), the difference between the activities of the two foils is a measure of the thermal neutron flux.

For technical reasons it was inexpedient to make these measurements in a mouse carcass. Instead, a presdwood phantom mouse weighing 23 grams, which had been prepared for measurements of soft X ray absorption,\(^{(6)}\) was utilized. Such a phantom has about 60% as many hydrogen and nitrogen atoms as the mouse. It seemed unnecessary to approximate the composition of the mouse more closely for the purpose of this observation. Exposure was made in the chamber for 44 minutes, corresponding to the LD\(_{95}\)-30 days for the mouse under the same conditions.

The foils were monitored an hour or more after exposure to avoid including in the measurement Cu\(^{66}\) or short-lived contaminants. The activities at the end of irradiation were 3.34 c/s for the cadmium-shielded foil and 10.2 c/s for the unshielded one, corresponding under the conditions used to 5160 d/s from thermal activation and 7670 d/s from activation by the unshielded flux. From these values, corrected for foil and counter efficiency, it was found that not more than 2.78 x 10\(^5\) thermal neutrons (per cm\(^2\) per sec) could occur within the phantom which was exposed to a flux on the order of 6 x 10\(^7\) fast neutrons per cm\(^2\) per second.\(^{(1)}\) Thus the
slow neutron flux was less than 0.5% of the fast, and in view of the greater biological effectiveness of the latter, the slow neutrons were of negligible importance in these exposures.

It may be pointed out that thermalization would be a factor of greater importance in the exposure of larger animals.

ACKNOWLEDGEMENTS

It is a pleasure to thank Dr. H. H. Vogel, Jr. for his technical guidance with the exposures and W. E. Kisieleski for the use of his laboratory facilities. The writer is further indebted to Dr. H. E. Kubitschek for his generous instruction and active encouragement, to Dr. A. M. Brues for his collaboration and interest, and to Dr. L. D. Marinelli for his guidance on the interpretation of the data.

REFERENCES


INFLUENCE OF THE HYPOPHYSIS AND OF THE ADRENAL CORTEX UPON THE TISSUE MAST CELL OF THE HAMSTER

Douglas E. Smith, Yevette S. Lewis and Sally T. Hartig

We have found, in the rat, that hypophysectomy or administration of ACTH or cortisone elicits small increases in the number of abnormal (vacuolation and clumping of cytoplasmic granules) mast cells (ANL-5332, p. 33). Similar studies have been carried out in the adult Syrian hamster (100 gm). Adrenalectomy and hypophysectomy are without effect on either the total number of mast cells or the number of abnormal mast cells. Both ACTH and cortisone injection, however, elicit marked increases in the number of abnormal cells in the mesentery, skin, and cheek pouch (Table II-13). Preliminary experiments indicate that the ACTH effect does not occur in the absence of the adrenals.
### TABLE II-13

The Influence of Cortisone and ACTH upon Mast Cell Number in the Hamster

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of treatment (days)</th>
<th>Skin</th>
<th>Mesentery</th>
<th>Cheek Pouch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total no. of abnormal cells *</td>
<td>Total no. of abnormal cells</td>
<td>Total no. of abnormal cells</td>
</tr>
<tr>
<td>Cortisone - intact hamster</td>
<td>1</td>
<td>335**</td>
<td>261</td>
<td>675</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>496</td>
<td>188</td>
<td>601</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>325</td>
<td>205</td>
<td>735</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>347</td>
<td>203</td>
<td>606</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>500</td>
<td>283</td>
<td>634</td>
</tr>
<tr>
<td>200 mg/kg/day, subcutaneously</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>381</td>
<td>338</td>
<td>631</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>389</td>
<td>282</td>
<td>1128</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>566</td>
<td>254</td>
<td>1080</td>
</tr>
<tr>
<td>ACTH - intact hamster</td>
<td>1</td>
<td>329</td>
<td>452</td>
<td>948</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>330</td>
<td>387</td>
<td>1031</td>
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<td></td>
<td>3</td>
<td>275</td>
<td>287</td>
<td>743</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>340</td>
<td>331</td>
<td>668</td>
</tr>
<tr>
<td>400 I.U./kg/day, subcutaneously</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>451</td>
<td>313</td>
<td>824</td>
</tr>
</tbody>
</table>

* Per 2.028 mm² of tissue.

** Average of 3 animals per treatment group.
PYRESIS STUDIES

Marcia White Rosenthal and Joan F. Fried

A search is being made for a pyrogen which will cause a reproducible, significant increase in the body temperature of the rat with a minimum of side effects, so that the antipyretic properties of a number of related drugs, including salicylic acid and aurintricarboxylic acid, may be compared. Of the pyrogens so far tested a subcutaneously injected suspension of yeast has produced the highest fevers, averaging 0.8°C above the temperatures of saline controls. Piromen, a polysaccharide, nonprotein, nitrogenous extract of Pseudomonas, has produced some temperature rise and may prove more useful in that it is simpler chemically than the yeast suspension.

Preliminary studies on fever production and the normal temperature variation in our stock Sprague-Dawley female rats indicate 1) a normal daily rise in body temperature in the latter part of the morning, with a fairly constant temperature range throughout the rest of the working day; 2) a rise in temperature produced by subcutaneous injection or by notching of the ears; 3) no increase or decrease in the normal temperature range during the period of estrus. The first two findings emphasize that all studies of pyresis and antipyresis must be carefully and adequately controlled.
METHOD FOR THE PREPARATION OF 5'-METHYL- AND 5'-ETHYLTHIOADENOSINE

F. Schlenk

For studies on methionine formation and on the transfer of biochemical “one carbon units” substantial amounts of 5'-methylthioadenosine were required. It was found that this compound can be obtained by biosynthesis using yeast (Torulopsis utilis or Saccharomyces cerevisiae).

Methionine reacts with adenosine triphosphate in fermenting yeast in the following way:

\[
\text{Methionine} + \text{ATP} \rightarrow \text{S-Adenosylmethionine} + 3 \text{Phosphate;}
\]

\[
\text{S-Adenosylmethionine} \rightarrow \text{Methylthioadenosine} + \text{Homoserine}.
\]

Ethionine reacts in an analogous fashion yielding ethylthioadenosine. An excess of the requisite sulfur-containing amino acid is incorporated into the growth or fermentation medium of yeast and the nucleoside may be extracted from the cells after a suitable reaction time.

Few commercial yeasts are produced in a medium sufficiently rich in methionine to permit the isolation of satisfactory amounts of methylthioadenosine; ethylthioadenosine is not detectable in such material. It is necessary to subject the yeast to a period of growth and fermentation in the presence of an excess of methionine (procedure A) or to grow a suitable strain of yeast in a medium enriched with methionine or ethionine (procedure B). Procedure A is recommended for the isolation of methylthioadenosine. Procedure B is preferable for the isolation of ethylthioadenosine, because the absence of methionine in the growth medium keeps the formation of the methyl compound at a minimum; (B) is the method of choice if sulfur- or methyl-labeled nucleoside is desired, because the relative yield is higher than that obtained by method A.

The optimal concentration of DL-methionine was found to be 5 millimoles, of DL-ethionine, 3 millimoles, per liter of culture medium. Copious aeration has to be provided during the growth period. The yeast is collected by centrifugation and washed twice.

For isolation of the nucleoside the yeast is extracted with 2 volumes of boiling water for 20 minutes. After cooling and removal of cell debris the supernatant is concentrated to 1/20 of its volume. Undesirable material is removed from the viscous liquid by precipitation with 15 volumes of acetone and filtration. Next, the acetone is removed by distillation and the nucleoside is isolated by precipitation with phosphotungstic acid. The washed phosphotungstate is decomposed by treatment with \( \text{Ba(OH)}_2 \) and the excess of \( \text{Ba}^{++} \) is removed by \( \text{CO}_2 \). Crystals of the nucleoside are obtained from the
filtered and concentrated solution on storage at low temperature. For re-
crystallization a small amount of hot water is used.

5'-Methylthioadenosine: MP 213°; $E_m(260\text{m}_\mu) = 15,500$; mol. wt. 297.3
($C_{11}H_{15}O_{3}N_{5}S$); yield: 40 to 70 mg. per 100 gm. of yeast (moist weight).

5'-Ethylthioadenosine: MP 187°; $E_m(260\text{m}_\mu) = 15,500$; mol. wt. 311.4
($C_{12}H_{17}O_{3}N_{5}S$); yield: 30 to 50 mg. per 100 gm. of yeast (moist weight).
INDOLE-3-ACETIC ACID OXIDASE

Robert E. Stutz

The investigation of the indole-3-acetic acid oxidase system has been advanced along several lines (cf. ANL-5332).

1. Relation to Glycolic-Glyoxylic Acid Oxidase

Because of the great variation in oxygen uptake that could be obtained under various conditions, it seemed likely that a component either of a dehydrogenase or of a terminal oxidase was limiting. If the indole-3-acetic acid oxidase system contains one or more dehydrogenases, it would be reasonable to assume that hydrogen is transferred to a terminal oxidase such as the glycolic-glyoxylic acid cycle.\(^{(1,2,3)}\) When this hypothesis was tested by adding glycolic acid to the crude lupine leaf enzyme from 3- to 8-week-old plants, the lag in oxygen uptake was either shortened or eliminated (Figure II-24). Glycolic acid appeared to exert no effect on either the dialyzed or \((\text{NH}_4)_2\text{SO}_4\)-precipitated enzyme, which may indicate that the coupling with the glycolic-glyoxylic acid cycle was disturbed by the purification. The oxygen uptake curves obtained with the dialyzed enzyme, the \((\text{NH}_4)_2\text{SO}_4\)-precipitated enzyme, resuspended free of \(\text{NH}_3\), and the enzyme from etiolated lupines gave a straight line when the reciprocal of the remaining substrate concentration was plotted against time. The normal oxygen uptake curve of the crude enzyme from the older light-grown lupine leaves gave a straight line when the log of the remaining substrate concentration was plotted against time.

2. Ether-soluble Cofactor

The influence of the ether-soluble cofactor is shown in Figure II-24, where the lag period in oxygen uptake of the crude enzyme is increased by the addition of extra cofactor even though glycolic acid has been added. The varying response of the \((\text{NH}_4)_2\text{SO}_4\)-precipitated enzyme to the dialyzable factors is shown in Figure II-25. Although \(\text{Mn}^{++}\) markedly increased oxygen uptake, the addition of the ether extract of the dialyzate along with \(\text{Mn}^{++}\) further increases the activity, suggesting that the concentration of the ether-soluble cofactor controls the absolute oxygen uptake under these conditions.

The removal of the ether-soluble cofactor from the dialyzate shortens the lag in oxygen uptake induced by the addition of the dialyzate. Also the final oxygen uptake (of approximately 600 \(\mu\text{l.}\)) may be increased two to five times by the presence of the dialyzate or ether-extracted dialyzate as is shown in Figure II-25. Although the significance of the apparent kinetic difference in the oxygen uptake induced by the recombination with the various dialyzate fractions has not been shown, correlation with the products, mineral requirements, and the respiratory quotient is being sought.
Figure II-24 Influence of glycolic acid on the IAA oxidase from the leaves of 3 and 8-week-old lupines.
Figure II-25  The response of purified IAA oxidase to glycolic acid and other factors. The enzyme was precipitated with (NH₄)₂SO₄ (25-100% saturation).
In preliminary trials none of the following has been found to be effective as a substitute for the ether-soluble cofactor: DPN, TPN, flavin mononucleotide, ascorbic acid, methylene blue, 2,4-dichlorophenolindophenol, or 2-methyl-1-4-naphthoquinone.

3. Catalase and Light

The addition of catalase to the enzyme from lupines grown and prepared in the dark (or dim-red illumination) introduces a lag into the oxygen uptake as does also the addition of the ether-extracted dialyzate from the light-grown lupine leaves (Figure II-26). The lag-inducing effect of catalase is accentuated by citrate-phosphate buffer; in this experiment a lag of two hours was induced. Citrate-phosphate buffer (0.1 M, pH 6.5) also suppresses the total oxygen uptake. Neither total darkness nor light (ca. 600 f.c.) had any effect on the course of the oxygen uptake. Catalase, but not dialyzed or boiled catalase, has been found also to induce a lag in the oxygen uptake of the enzyme from light-grown lupine leaves.

4. Enzyme Fractionation

Preliminary experiments with (NH₄)₂SO₄-treated enzyme indicate that the fraction that precipitates between 25 and 100% saturation gives the maximum oxygen uptake; about half of the activity is obtained in the 50-75% saturation fraction. Although the enzyme has not yet been prepared completely free of the ether-soluble cofactor, both the total oxygen uptake and the form of the oxygen uptake curves depend on the concentrations of the dialyzable cofactors or inhibitors (Figure II-25).

REFERENCES


Figure II-26 The response of IAA oxidase from etiolated lupines to light, catalase, and buffers. All manipulations of the enzyme from 10-day-old seedlings were carried out under dim-red illumination.
THE BEHAVIOR OF RIBONUCLEASE IN STRONG ACID MEDIA, BIOLOGICAL AND PHYSICAL PROPERTIES OF RECOVERED MATERIAL

A. L. Koch, W. A. Lamont, and J. J. Katz*

Ribonuclease has been dissolved and recovered from a variety of anhydrous strong acids. In most cases material possessing considerable ribonuclease activity was recovered, particularly if the temperature was kept low and if the time of exposure was moderately short. The solvents used were anhydrous hydrogen fluoride, trifluoroacetic acid, sulphur dioxide-hydrogen fluoride, and formic acid.

1. Sedimentation Characteristics

All samples were dissolved (after complete removal of the solvent) in 0.1 M NaCl to make solutions approximately 0.5 per cent in protein. The actual amount of protein was determined in each case either by the area of the schlieren diagram or by the ultraviolet absorption at 280 m\(\mu\), or both. Neither of these procedures is precise. In the first case, the inherent accuracy of the method is low, and in the latter case, presence of trace metals effects the spectra of the solutions, as is shown by the fact that the ratio of extinction at 280 m\(\mu\) to that at 260 m\(\mu\) is not rigorously constant. We feel that \(\pm 10\) per cent represents the precision of these measurements.

The solutions were unbuffered, and no attempt was made to control pH except in one run of a sample that had been dissolved in trifluoroacetic acid (#179) where the pH was raised from 3 to 4.3. The remainder of the samples in solution had pH's between 4.3 and 4.7 as determined by indicator paper. The samples were centrifuged at top speed (59,780 RPM) in the model E analytical ultracentrifuge. The samples were run at approximately 20\(^\circ\)C although room temperature was in the vicinity of 28\(^\circ\)C. The high room temperature leads to increased errors in temperature measurement.

The photographic plates were measured in a microcomparator, and the least-squares regression taken of the logarithm of the distance of the boundary maximum to the center of rotation. The sedimentation constant was computed from the slope and from the speed of rotation. This value was then corrected to 20\(^\circ\)C and pure water solution.\(^{(1, 2)}\) This was accomplished by interpolating the temperature for the middle of the photographic run from the initial and final temperature measurements and then subtracting one degree\(^{(3)}\) to correct for the cooling effect resulting from the stretching of the rotor under the high gravitational field. From known physical properties of the solvent (viscosity and density) at this temperature and the partial specific volume of ribonuclease, the values of \(S_{20,w}\) were calculated, and are presented in Table II-14 for samples exhibiting considerable activity.

* Chemistry Division
TABLE II-14

Sedimentation Constants of Ribonuclease Samples

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Treatment</th>
<th>$S_{20,w}$ (10^{-13} per sec.)</th>
<th>Protein conc. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>166</td>
<td>None</td>
<td>1.86</td>
<td>0.31</td>
</tr>
<tr>
<td>A</td>
<td>None</td>
<td>1.90</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.93</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.73</td>
<td>0.12</td>
</tr>
<tr>
<td>176</td>
<td>HF</td>
<td>1.84</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.87</td>
<td>0.21</td>
</tr>
<tr>
<td>177</td>
<td>HF</td>
<td>1.88</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.89</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.80</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.69</td>
<td>0.20</td>
</tr>
<tr>
<td>178</td>
<td>HF</td>
<td>1.89</td>
<td>0.39</td>
</tr>
<tr>
<td>179</td>
<td>Trifluoroacetic Acid</td>
<td>1.85</td>
<td>0.26</td>
</tr>
<tr>
<td>180</td>
<td>SO$_2$</td>
<td>1.76</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.76</td>
<td>0.26</td>
</tr>
<tr>
<td>181</td>
<td>SO$_2$</td>
<td>1.84</td>
<td>0.45</td>
</tr>
<tr>
<td>201</td>
<td>HCOOH</td>
<td>1.89</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Several of the preparations were examined at a variety of concentrations. It is generally expected that the sedimentation constant should rise with decreasing concentrations; however, we have repeatedly observed that at concentrations less than about 0.2 per cent there is a considerable reduction in the sedimentation constant. There are two possibilities for this result. The first is that, under these conditions, the boundary is unstable and convection stirring reduces the apparent sedimentation constant; the second, that ribonuclease in concentrated solution is mainly present in the form of a dimer, which dissociates at reduced concentrations. We believe that the former is the correct interpretation.

The effects of convection should be more apparent when a) the temperature of the rotor is rising rapidly, b) long periods of time are used, and c) the protein concentration is small, thus reducing the stabilization due to the density gradient. These are just the conditions of these runs. If the first possibility is the correct one, then the apparent diffusion constant,
as estimated from the rate of flattening of the peak, should be increased. This appears to be the case as determined from visual inspection of the photographs from the runs in which the sedimentation constant is reduced.

The other alternative, that ribonuclease is in reality a smaller molecule (m.w. 7,000) which dimerizes in concentrated solution, is rejected on the basis of runs at even lower concentrations in the synthetic boundary cell. The use of this apparatus allows the measurement of $S_{20,w}$ with smaller concentrations and shorter times, because a boundary is produced that is initially very sharp and therefore easily measurable. The $S_{20,w}$ values are listed below in Table II-15.

**TABLE II-15**

Sedimentation Constants at Low Protein Concentration
Measured with the Synthetic Boundary Cell.
(Ribonuclease prep. R 531 Worthington)

<table>
<thead>
<tr>
<th>Protein concentration (per cent)</th>
<th>$S_{20,w}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>2.18</td>
</tr>
<tr>
<td>0.10</td>
<td>2.17</td>
</tr>
<tr>
<td>0.20</td>
<td>2.12</td>
</tr>
<tr>
<td>0.20</td>
<td>2.12</td>
</tr>
</tbody>
</table>

A run was also made at the 0.2 per cent level in the ordinary cell and an $S_{20,w}$ of 1.92 obtained. Thus it is apparent that decreasing the protein concentration 4-fold does not appreciably decrease the sedimentation constant.

Several theories have been presented to explain the dependence of sedimentation constant upon protein concentration. For small symmetrical molecules at relatively low concentration the following relation would appear to be appropriate:

$$S = S_0 - 6.875 (S\phi)$$

where $S_0$ is the sedimentation constant at infinite dilution and $\phi$ is the volume fraction of protein. For the average conditions of those runs with active enzymes the correction amounts to 0.04.
The average $S_{20,w}$ obtained in all runs, excluding those of samples #167, 180 and 200, which are significantly lower, is $1.873 \pm 0.028$. Only one-half of the standard error is due to errors in measurement of the photographic plates, the remainder reflecting errors in temperature measurement. We can therefore conclude that $S_{20,w}$ of native ribonuclease is 1.91.

Sample #167 had a very significantly decreased sedimentation constant ($S_{20,w} = 1.64$). This preparation had been treated with HF at $0^\circ$ for two hours and possessed only very small biological activity. In this run as in all others, only one component was detected, and it is concluded that the decrease is a result of the unfolding of the peptide chain of the molecule. If we assume that the weight of the molecule and its degree of hydration are unchanged by the denaturing process, we may write:

$$\frac{f_d}{f_n} = \frac{S_n}{S_d} = \frac{1.87}{1.64} = 1.14$$

where the "f's" refer to frictional coefficients, and the subscripts "d" and "n" refer to the denatured and native states. Since the frictional coefficient of ribonuclease is 1.04 times that of the equivalent sphere, it is apparent that the frictional coefficient of the denatured molecule is 1.19 times that of the spherical molecule. In terms of shape(4) this would mean that, if the original molecule were approximately spherical, the denatured molecule is four times as long as it is wide.

In order to study this phenomenon further we prepared another sample of ribonuclease that had been HF-denatured (Table II-16). The last run in the table was done, not in 0.1 M NaCl, but in 0.1 M NaCl plus phosphate buffer, to bring the pH to 7.0. The average sedimentation constant was $1.67 \pm 0.03$, which corresponds to a molecule which is three times as long as it is wide.

**TABLE II-16**

The Sedimentation Constant of HF-Denatured Ribonuclease (Worthington prep. R 531, sample #200)

<table>
<thead>
<tr>
<th>Protein concentration (per cent)</th>
<th>$S_{20,w}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.66</td>
</tr>
<tr>
<td>0.5</td>
<td>1.61</td>
</tr>
<tr>
<td>0.4</td>
<td>1.71</td>
</tr>
<tr>
<td>0.3</td>
<td>1.65</td>
</tr>
<tr>
<td>0.3</td>
<td>1.70</td>
</tr>
</tbody>
</table>
Sample 180 which had been dissolved in liquid $SO_2$ for five hours at $0^\circ C$ possessed considerable biological activity, but, for some reason that we do not understand, had a significantly reduced sedimentation constant.

2. Biological Activity

Ribonuclease activity has been measured by a number of the changes it produces in the properties of a ribonucleic acid solution. We have utilized the method of Kunitz,$^5$ which depends on the fact that native ribonucleic acid absorbs less light at pH 5 and 300 m$\mu$ than does the reaction product. Kunitz assumed that the hydrolysis reaction was first order, and therefore plotted the logarithm of the difference between the extinction of the solution and the final value against time. He then considered the slope to be proportional to enzymatic activity. We find that the plots so obtained are concave upward and that the final slope is independent of enzyme concentration. Therefore, the initial slope has been taken to be proportional to enzyme activity.

In addition to the difficulties attendant on the estimation of the initial slope, it was found that the measurement of the small changes in optical density in these quite dense solutions is subject to considerable variation. Therefore we used, as a blank, a solution that has approximately the final optical density. A solution of thymine in alkali has proved satisfactory.

Although ribonuclease is a particularly hardy enzyme with no known metal cofactors, it is apparently inhibited by trace amounts of heavy metals and, since these are present in our reagents, and are introduced as well during the treatment of the enzymes in the all-metal vacuum lines employed, we have found it necessary to carry out assays in the presence of 0.25% bovine serum albumin (BSA). The increase in activity when BSA is present is particularly noticeable when denatured preparations are assayed. For instance, sample 200 has only 0.1 per cent the activity of the control in the absence of BSA and 12 per cent in its presence - a hundred-fold increase. The activities of the controls are only increased by a factor of two or three.

An additional complication arises in the case of those preparations that have been almost completely denatured. If the concentration of protein is increased in order to obtain a satisfactory rate of decrease of optical density, the solution becomes turbid, presumably as the result of the formation of a complex between the denatured protein and RNA. In time the turbidity decreases, presumably due to the action of residual ribonuclease activity. We have therefore assayed such solutions using protein concentrations low enough to avoid this and using sufficiently long periods of time, meanwhile maintaining the temperature at 27°C.

Considering all these factors together, as well as the uncertainty in the estimation of protein concentration, we estimate that the relative activity of the preparation is obtained with about 20 per cent accuracy.
A summary of the treatments and resultant activity is indicated in Table II-17. In general it is seen that increasing temperature and increasing time lead to a decreased activity, but active preparations may be obtained from all solvents tested.

**TABLE II-17**

The Biological Activity of Recovered Ribonuclease Samples

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Time (hours)</th>
<th>Temperature (°C)</th>
<th>Relative Activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>176†</td>
<td>1.5</td>
<td>-80</td>
<td>80</td>
</tr>
<tr>
<td>178</td>
<td>1</td>
<td>-80</td>
<td>100</td>
</tr>
<tr>
<td>177</td>
<td>5</td>
<td>-80</td>
<td>43</td>
</tr>
<tr>
<td>167</td>
<td>2</td>
<td>0</td>
<td>0.4***</td>
</tr>
<tr>
<td>200</td>
<td>3</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.14***</td>
</tr>
<tr>
<td><strong>Trifluoroacetic acid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>179</td>
<td>2</td>
<td>-20</td>
<td>45</td>
</tr>
<tr>
<td>192</td>
<td>2</td>
<td>25</td>
<td>60</td>
</tr>
<tr>
<td>193*</td>
<td>2</td>
<td>25</td>
<td>0.6***</td>
</tr>
<tr>
<td>202**</td>
<td>2</td>
<td>25</td>
<td>110</td>
</tr>
<tr>
<td><strong>SO₂ + HF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>181</td>
<td>1</td>
<td>-80</td>
<td>64</td>
</tr>
<tr>
<td>180</td>
<td>1.25</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td><strong>Formic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>201</td>
<td>1.5</td>
<td>25</td>
<td>70</td>
</tr>
<tr>
<td>189</td>
<td>2</td>
<td>25</td>
<td>20</td>
</tr>
</tbody>
</table>

† It was noted during this run that the solution was too concentrated and that ribonuclease crystallized out of solution.

* In this run trifluoroacetic anhydride was present.

** Equal amounts of BSA and ribonuclease were present.

*** These assays were run in the absence of bovine serum albumin.
All samples were water-soluble except #189 and #193. The former was quite inactive although its replicate #201, was soluble and quite active. Possibly this is the result of trace contamination of the formic acid with water. In the latter case trifluoroacetic anhydride was present and no doubt fluoroacetylated the protein. This material, when dissolved in dilute alkali, lost the yellow color characteristic of protein treated with trifluoroacetic acid. Upon neutralization the material precipitated, but could be redissolved by the addition of more acid. The alkali treatment removed the color, but diminished to zero the activity of the recovered material. It should be mentioned that the isoelectric point of native ribonuclease is about 7.8 and that the original material was insoluble at a pH of 6. The addition of BSA ameliorates the destructive action of trifluoroacetic acid, as indicated in experiment 202.

In conclusion, then, it is possible to dissolve ribonuclease in a variety of anhydrous media and recover a water-soluble, active preparation. Upon extended treatment with HF it is possible to uncoil the molecule with loss of biological activity.

REFERENCES


DISTRIBUTION OF ISOTOPE IN THE PURINES OF ESCHERICHIA COLI
AFTER GROWTH IN THE PRESENCE OF VARIOUS
RADIOACTIVE PRECURSORS

Arthur L. Koch

During the course of several years, strain B/1.5 of Escherichia coli has been grown in synthetic media containing a variety of labeled potential purine precursors. The purines of the bacteria were isolated in chromatographically pure form, and the guanine (as representative of the purines of the cell)(1) degraded carbon by carbon. The results of these experiments are summarized in Table II-18.

TABLE II-18

Distribution of C\(^{14}\) in the Purine Nucleus
(As % Total Activity in the Purine Molecule)

<table>
<thead>
<tr>
<th>Labeled Precursor</th>
<th>CO(_2)</th>
<th>Formate</th>
<th>2-C(^{14})-glycine</th>
<th>d,1-3-C(^{14})-serine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon #</td>
<td>Aerobic</td>
<td>Anaerobic</td>
<td>Initial</td>
<td>Terminal</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3.5</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>94</td>
<td>98</td>
<td>15.6</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>2</td>
<td>79.7</td>
<td>76</td>
</tr>
</tbody>
</table>

The guanine was degraded by the methods of Heindrich and Wilson(2). In the procedure the guanine molecule is oxidized by permanganate, yielding the 8 carbon as urea and the 2 carbon as guanidime. A separate sample of guanine is hydrolyzed with strong acid to yield the 4 and 5 carbons as glycine, which may be degraded further if necessary. The activity in the 6 position is computed by difference. In several of the experiments it was not necessary to carry out the complete degradation because of other data available from the growth experiment.

From the data presented in Table II-18, it is apparent that CO\(_2\) is utilized for the synthesis of only the 6 position of the purine ring. The activity listed for the 8 position is probably a result of contamination of the urea sample with CO\(_2\) released from the 6 position by permanganate
oxidation. In the other experiments precautions were taken to eliminate completely CO₂ from the urea samples. The fact that CO₂ is not effectively utilized for either the 2 or the 8 position is indicative of the lack of ability of these organisms to reduce CO₂ to the level of the postulated one-carbon fragments used for the synthesis of these positions. Even the conditions of anaerobiosis which should favor such reduction do not increase the incorporation into the 2 and 8 positions.

Two experiments were done with formate as the precursor: in the first, tracer was added at the time of inoculation; in the second, it was added near the end of the growth period. The distribution of isotope was the same in both cases.

The second experiment was performed in order to find out if the results of the first experiment were due to the phenomenon discovered by Buchanan and Schulman. These authors found conditions in which the 2 position of the purine ring was labile and formate activity could be introduced into the intact purine ring.

The fact that activity is not found in the 2 position in either case indicates that formate is not a precursor of this position. In other organisms that have been studied formate is used equally for the synthesis of the 2 and 8 positions. The activity in the 6 position is almost certainly derived from the fixation of CO₂ produced by the action of formic dehydrogenase on the formic acid. Not only is the majority of the formate added converted to CO₂, but the activities in other compounds of the cell (Kreb's cycle compounds, amino acids, and pyrimidines), which are known to be produced by CO₂ fixation, are of the order of magnitude of the activity in the 6 position. Formate is neither an effective precursor of the glycine moiety of the purines nor is it utilized for the synthesis of bacterial protein glycine and serine.

At low levels, 2-¹⁴C-glycine is used almost exclusively for the synthesis of glycine and serine of the protein and for the glycine moiety of the purine ring. Glycine is used to a limited extent for the synthesis of the 8 position. If glycine is present in great excess in the medium, however, it is used almost equally for the synthesis of the 2, 5, and 8 positions. In this experiment it was not necessary to degrade the guanine, since the distribution could be inferred from the fact that 2.86 moles of the alpha carbon of glycine were used for the synthesis of one mole of guanine, and the CO₂ produced by the culture had very little radioactivity.

Another effective precursor of the 8 position is d₁-³-¹⁴C-serine. No activity is present in the 2 position, and the activity of the 6 position should be small because only 3 per cent of the activity of the serine is converted to CO₂. Although the 4 and 5 carbons have not been isolated, the protein glycine contains very little activity. Therefore, essentially all the activity of the molecule should reside in the 8 position. However, the
specific activity associated with the 8 position as determined from the isotope content of the isolated urea was about 50 per cent of that expected from the specific activity of the molecule as a whole. This is probably due to the production of unlabeled urea molecules which would dilute the activity of the urea derived from the 8 position. These urea molecules could come either from the 4 position during the permanganate oxidation or from the 2 position, if some xanthine were present in the guanine sample. Although this point must be investigated further, we shall assume, for the purposes of this paper, that all of the activity of the molecule is in the 8 position.

From the distribution data and other data from the same or similar experiments, it is possible to calculate the percentage of carbon atoms of a particular position which were derived from the precursor.

In the experiment in which glycine was present in large amounts in the medium, it can be assumed that the concentration and specific activity remained constant during the growth of the culture. This makes it possible to calculate, from the specific activity of the isolated guanine and a knowledge of the extent of growth during the experimental period, that about 3 moles of glycine are utilized per mole of guanine synthesized. From that information it can be concluded that the 2, 5, and 8 positions must be almost solely derived from the alpha carbon.

In experiments in which glycine or serine are present in small amounts, the number of moles of glycine utilized can be calculated, if it is known that the compound is removed by the bacteria at a rate that is concentration-independent, and the bacteria are not continuously diluting the precursor in the medium by synthesis.

From the accompanying paper,(6) it can be seen that the above conditions are met in the case of glycine. Thus, if the experiments are completed before the isotope is removed completely from the medium, we may proceed as before.

There is however, another method of determining the amount of precursor used for the synthesis of a given position of the purine ring. From the kinetics of isotope uptake, one can compute the number of moles used in the synthesis of a new bacterium; from the partition of the bacteria one may then compute the number of moles of precursor used for the synthesis of a purine molecule in the bacterium; and, finally, from the distribution of activity within the purine molecule, one may calculate the moles of precursor used for the synthesis of a particular carbon.

Using either of these methods it was found that 1.0 mole of glycine alpha carbon was used for the synthesis of the 5 position of the purine ring, and about 0.3 for the synthesis of the 8 position. With the d,1-3-C14-serine it was found that 1.0 mole of the tracer was used for the synthesis of the 8 position.
When CO₂ was used as the labeled precursor, the determination was complicated by the production of unlabeled CO₂ from lactic acid or glucose, which were the main carbon sources for the growth of the bacteria. It can be shown, however, that in a sealed system where growth is exponential, the average of the initial and final specific activities given by

\[ x = x_f \ln \frac{x_i}{x_f} + x_f \]  

is the value to be expected in a compound that is solely derived from CO₂. The observed radioactivities correspond to 77-80 per cent of this value. This value is a minimal one: the intracellular specific activity of CO₂ at any time should be less than the extracellular value, because of the intracellular production of unlabeled CO₂.

**SUMMARY**

In growing cultures of *E. coli*, CO₂ is utilized solely for the synthesis of the 6 carbon of the purine. Formate per se and the beta carbon of serine are used for the 8 position. The alpha carbon of glycine is used primarily for the 5 position, secondarily for the 8, and, at high concentrations, also for the synthesis of the 2 position.

These results are similar to those obtained with other organisms, except for the observation that formate and serine are not good precursors of the 2 position. For this reason, this system offers the possibility of distinguishing between the two types of one-carbon fragments that are utilized for the two ureide carbons of the purine ring.

Both glycine and serine appear to be utilized immediately and solely for the synthesis of the purine ring. Both act to stop completely or almost completely the de novo synthesis of the particular positions from the major carbon source of the medium.

**ACKNOWLEDGEMENTS**

I wish to thank Verne Hospelhorne, H. Richard Levy, William A. Lamont, and Dorothy M. Tahara for assistance in various phases of this investigation. I am particularly indebted to Dr. Robert W. Swick for advice and assistance in carrying out some of the degradations.
REFERENCES


Plant tissues oxidize tryptophan to the growth hormone indoleacetic acid (IAA). The enzymes involved have not yet been described biochemically. Characterization of these enzymes is of particular interest in view of their sensitivity to ionizing radiation. Previous reports (ANL-5288 and ANL-5332) indicate that the enzyme complex is a nonparticulate component of the cytoplasm of leaf cells. Further localization within the cytoplasm and enrichment of the enzyme has now been achieved by the use of ultracentrifugation.

Leaves and buds of young mung bean plants were frozen in liquid nitrogen and dispersed in twice their weight of water. (Dispensions with water, final pH 6.2, were more active than neutral dispersions made with 0.1 or 0.5 M phosphate buffers.) Preliminary examination in the analytical ultracentrifuge indicated the presence of essentially two fractions: a fast-moving component with an $S_{20}'$ of about 18 svedberg units, and a slower-moving, heterogeneous component containing substances with $S'$ values ranging from 0 to about 4. Dispersions were, therefore, first centrifuged at 105,000 $\times g$ for 30 minutes to remove particulate components. The resultant supernatant was transferred to a pair of tubes. These were again centrifuged for 200 minutes at the same speed to throw down a relatively clean preparation of the fast-moving component. In one tube the pellet and supernatant were redispersed ("reconstituted"). The pellet and supernatant in the other tube were separated for analysis. Two runs were made with 7-day and 15-day mung bean seedlings. With the older seedlings, the pellet was again recycled at 101,000 $\times g$ for 10 minutes and 240 minutes. All fractions were made to equal volumes, 0.01 in ionic strength. Sedimentation patterns of the various preparations are shown in the figure. Enzymatic activities (rates of IAA formation from tryptophan at pH 7.4, 30°C) and protein concentrations are given in the table. From the table and figure it may be concluded that:

1. The cytoplasmic proteins of mung bean leaves fall into two major components ultracentrifugally distinguishable. One component is homogenous in the ultracentrifuge, with an $S_{20}'$ of about 19, and comprises over 50 per cent of the cytoplasmic protein. The remaining component is heterogeneous, composed of substances with $S_{20}'$ of 4 or less, and not resolvable with the techniques used.

2. The enzyme system involved in the conversion of tryptophan to IAA is chiefly in the heterogeneous fraction. However, about 12-14 per cent of the enzyme activity occurs associated with the $S_{20}'$ 19 component. If this activity derives from enzymes adsorbed on the heavy protein, elution is not accomplished by recycling at neutral pH and at an ionic strength of 0.01.
<table>
<thead>
<tr>
<th>Fraction</th>
<th>IAA formed per min. per ml enzyme $\mu$g $x 10^{-3}$</th>
<th>Protein conc.$^1$ mg/ml enzyme</th>
<th>$\mu$g $x 10^{-3}$ IAA formed per min. per mg protein</th>
<th>Apparent protein conc.$^2$ mg per ml enzyme</th>
<th>S' of fast component</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>7-day plants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IA-Reconstituted</td>
<td>25</td>
<td>4.25</td>
<td>5.9</td>
<td>2.4</td>
<td>17.5</td>
</tr>
<tr>
<td>IB-Supernatant</td>
<td>29</td>
<td>1.88</td>
<td>16</td>
<td>3.1</td>
<td>17.7</td>
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<tr>
<td>IC-Pellet</td>
<td>3.9</td>
<td>1.88</td>
<td>2.1</td>
<td>0</td>
<td>18.1</td>
</tr>
<tr>
<td><strong>15-day plants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIA-Reconstituted</td>
<td>46</td>
<td>2.88</td>
<td>15</td>
<td>3.2</td>
<td>18.6</td>
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<tr>
<td>IIB-Supernatant</td>
<td>41</td>
<td>1.59</td>
<td>26</td>
<td>3.5</td>
<td>19.2</td>
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<tr>
<td>IIC-Pellet</td>
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<td>18.8</td>
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<tr>
<td><strong>Recycle</strong></td>
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<tr>
<td>IIC-1 Pellet (10')</td>
<td>1.3</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>IIC-2 Pellet (240')</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIC-3 Supernatant (240')</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$TCA-precipitable N x 6.25

$^2$Calculated from peak areas on sedimentation plates
3. It may be suggested that the higher values for protein concentration as calculated from peak areas on the photographic plates are caused by non-protein colloids. If so, the latter are probably associated chiefly with the S' 0-4 fraction, since the pellet alone, with no photographically perceptible slow-moving contaminant, did not show protein values by area determination higher than values obtained by direct nitrogen analysis.

Figure II-27 Sedimentation patterns of cytoplasmic fractions of mung bean leaves. Spinco Model E analytical ultracentrifuge; 59,780 r.p.m. The numbers in parentheses represent the minutes to exposure.
THE STABILITY OF DNA DURING GROWTH

Robert W. Swick, Dorothy M. Tahara and Arthur L. Koch

The “half-life” of DNA in the livers of adult rats has been estimated to be at least 70 days in experiments wherein the animals were continuously exposed to $^{14}$O$_2$ for periods long enough to insure isotopic saturation of the liver. It appears probable that this represents, not true turnover, but replacement of dead cells, and leaves unanswered the question of the stability of DNA during growth.

In order to measure the stability of DNA in an active mitotic state, weanling rats were similarly treated with isotopic CO$_2$ until their body weights had doubled (9 days). It was calculated that 55% of the final liver weight had been formed during exposure, i.e., on the average, every cell in the liver had divided once. Hepatic DNA and RNA purines were isolated by salt extraction, followed by hydrolysis of RNA at pH 13 and hydrolysis of DNA at pH 3 at 100°C to yield apurinic acid. Adenine and guanine were isolated by ion-exchange and purified through the silver salts. The specific activity (s.a.) of the C-6 position of RNA adenine was 81% of the s.a. of body CO$_2$ as determined by the s.a. of urea, i.e., liver RNA adenine had been almost completely replaced during the experiment. However, the s.a. of DNA adenine was only 44% of that of urea or 54% of that of RNA adenine. Because RNA appears to be undergoing rapid turnover even during growth, the s.a. of RNA adenine probably reflects the s.a. of the “purine precursor pool” from which adenine is drawn for DNA synthesis. Since the s.a. of DNA adenine bears the same relation to the s.a. of RNA adenine that new liver bears to the total liver, one concludes that only one cell complement of DNA has been synthesized de novo during mitosis and that the original DNA has been retained. These results contradict those of Stevens(1) and of other workers,(2) who found that the rate of incorporation of $^{32}$P into DNA was twice the mitotic index.

The stability of DNA during growth was demonstrated in another way: two rats were exposed to $^{14}$O$_2$ for one week after which one (I) was killed and the other (II) was removed from isotope until it had doubled its weight (19 days). The s.a. of RNA adenine was 75% that of urea in I, but only 6% in II, as expected. On the other hand, the s.a. of DNA adenine was 27% that of urea in I and 25% in II. Since the s.a. of the DNA adenine in II approximated an average of the initial s.a. of DNA adenine (calculated from I) and of the estimated average s.a. of the “purine precursor pool” (in II), which was derived from the turnover of RNA during the second period, one concludes again that the DNA present in the liver was retained during growth.
REFERENCES


TRYPTOPHAN PEROXIDASE AND ARGINASE IN REGENERATING LIVER

John F. Thomson and Eleanor M. Moss

Since the X-irradiation-induced increase in rat liver tryptophan peroxidase (TPO) activity is presumably mediated through the adrenal cortex (the increase does not occur in adrenalectomized animals), it was of interest to study the effects of adrenalectomy on the levels of TPO in regenerating rat liver. We are also including data on arginase activity in adrenalectomized and non-adrenalectomized rats after partial hepatectomy.

The data on TPO activity in regenerating liver are shown in Figure II-28. These results are very interesting in that, unlike most other enzymes which have been studied, the activity of TPO on a dry weight basis did not return to normal levels until 7-10 days after operation. The capacity for the system to "adapt" to large amounts of tryptophan was even further impaired. Differences between adrenalectomized and non-adrenalectomized rats were most marked on the first two days after operation. The high value in the normal rats on the first day probably reflects an adrenal response to trauma which persisted for 24 hours. We are not able to explain the greater activity in the livers of adrenalectomized rats on the second postoperative day.

In the case of arginase, on the other hand, the activities (measured by the method of Roberts) decreased only 13 per cent on the first day after operation, and returned to normal within 4 days in both adrenalectomized and non-adrenalectomized rats. The control values for adrenalectomized rats were 20 per cent lower than those for non-adrenalectomized controls.

REFERENCES


Figure II-28 Tryptophan peroxidase activity is regenerating rat liver. Three to six rats were used for each point, except the controls (zero time). The control activity averages were 8.27 μM kynurenine formed/hr./g. dry weight, for 43 non-adrenalectomized rats; 6.80 for 30 adrenalectomized rats; and 121.3 for 12 non-adrenalectomized rats given 2 g./kg. DL-tryptophan intraperitoneally 6 hours prior to sacrifice. All rats were Sprague-Dawley females weighing 180-220 gm. Adrenalectomy was performed 5 days before hepatectomy; the rats were maintained on 1 per cent saline and daily administrations of DOCA, 1 mg./kg.
HEMOLYSIN PRODUCTION IN X-IRRADIATED RABBITS

B. N. Jaroslow and W. H. Taliaferro

The decreased ability of X-irradiated rabbits to produce hemolysins to sheep red cells has been well established. In a previous Quarterly Report (ANL-5288) we reported that mixing the antigen for 10 minutes with homogenized rabbit spleen prior to injection enabled X-irradiated (400 r) rabbits to produce hemolysin titers within the normal range. The major difference between the unirradiated and irradiated animals was the longer induction period in the latter group. Since then we have used mouse spleen, mouse ascites tumor or Hela cell tissue cultures in place of rabbit spleen. In each instance a marked increase in hemolysin production in irradiated rabbits has been noted. The work is still in its preliminary stages and a full analysis of the results attends the accumulation of more data.

THE RELATIONSHIPS BETWEEN NUCLEOLAR AND NUCLEAR GROWTH AND FORM DURING DEVELOPMENT IN PARAMECIUM

C. F. Ehret and E. L. Powers

The development of the macronucleus and nucleolus has been studied in exconjugants of Paramecium bursaria following formation of the zygote nucleus, and by means of phase contrast and electron microscopy. Eleven morphologically distinct stages in macronuclear development have been recognized, beginning with the micronucleus-like microform and terminating in the vegetative interphase form (Figure II-29). The average time course of these events is given in Figure II-30. Within the macronuclear anlagen at least three waves of nucleoli (nucleolar generations) have been observed, during anlage stages 3, 5, and 8 respectively. Nucleolar extrusion from the macronuclear anlagen occurs during stages 6 and 9; the nucleoli extruded are those of the second and third nucleolar generations respectively. Nucleolar generation and extrusion from the old macronucleus is synchronous with that observed in the anlagen. Unextruded nucleoli enlarge by growth and by aggregation within the macronucleus. Later coalescence of nucleoli is associated with development of a net-like structure which disappears from the nucleus with an ensuing nucleolar generation. A similar nucleolar cycle of generation, enlargement, and net-like coalescence is observed during vegetative fission, beginning prior to cytokinesis and during micronuclear prophase-metaphase. The young nucleoli at the time of extrusion are isomorphic with cytoplasmic particulates previously described as mitochondria. (1)

REFERENCES

Figure II-29 A generalized description of the stages of macronuclear development: 1-microform; 2-swollen form; 3-first nucleolar generation; 4-nucleolar enlargement; 5-second nucleolar generation; 6-nucleolar extrusion; 7-nucleolar enlargement; 8-third nucleolar generation; 9-nucleolar extrusion; 10-nucleolar enlargement; (11-vegetative interphase).

Figure II-30 The relationship between stage of macronuclear anlage and time at which observation was made, as measured from conjugant-separation time. Average conjugation time was 24.1 hours for Variety 1 (B x D) animals at 25°C. The encircled figures refer to the numbers of animals observed at each point.
CLEAVAGE TIME IN X-IRRADIATED ASCARIS EGGS

Theodore N. Tahmisian, Rosemarie L. Devine and Betty Jean Wright

We are presently investigating the effect of X-irradiation on the cleavage time of Ascaris eggs. Eggs were irradiated at doses varying from 2.5 Kr to 7500 Kr. The results have been approximated as follows:

<table>
<thead>
<tr>
<th>DOSAGE</th>
<th>EXTENT OF MATURATION</th>
<th>% OF TOTAL COUNT</th>
<th>LENGTH OF TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Worm larval</td>
<td>80%</td>
<td>2 days</td>
</tr>
<tr>
<td>2.5 Kr</td>
<td>Worm larval</td>
<td>27%</td>
<td>3 days</td>
</tr>
<tr>
<td>5 Kr</td>
<td>Worm larval</td>
<td>2%</td>
<td>3 days</td>
</tr>
<tr>
<td>20-500 Kr</td>
<td>Multi-cellular</td>
<td>95%</td>
<td>3 days</td>
</tr>
<tr>
<td>1000 Kr</td>
<td>Multi-cellular</td>
<td>8%</td>
<td>9 days</td>
</tr>
</tbody>
</table>

With doses exceeding 5 Kr, development progressed only as far as the multi-cellular stage and no worm larvae were formed. At 4000 Kr to 7500 Kr no cleavage occurred, even after nine days of incubation.

It has been reported that cold storage (0°-5° C.) of 5 Kr irradiated Ascaris eggs increases the possibility of recovery, allowing a greater percentage of the eggs to develop to the embryonic or worm-larval stage. (1) In initial studies we did not find this to occur. However, we are now carrying on further experiments to determine if cold treatment following irradiation at various dosages will afford sufficient protection to permit a significant increase in development beyond that reported above.

REFERENCE

STUDIES ON THE ROLE OF THE NUCLEUS IN PROTEIN SYNTHESIS

A. D. Barton and A. K. Laird

In a previous study of the intracellular changes in protein and nucleic acids in rat liver during the rapid synthesis of protein which accompanies refeeding after starvation,\(^1\) it was found that both the nucleus and the cytoplasm contribute to the formation of new protein; the cytoplasmic protein accumulated in a straight-line fashion throughout the recovery period, while from time to time the nucleus apparently accumulated protein and then released it to the cytoplasm. These biochemical changes were accompanied by cytological evidence of nuclear activity in terms of changes in nuclear size and nuclear outline, the nuclei being wrinkled and smaller at times when protein was apparently released to the cytoplasm. In a study of the effects of thioacetamide on nuclear protein and nucleic acids,\(^2\) it was found that this drug induces a rapid accumulation of protein and ribonucleic acid, first in the nucleus and later in the non-sedimentable fraction. These changes suggest that thioacetamide may act on the same mechanism for nuclear synthesis of protein that was demonstrated in the experiments on protein synthesis during refeeding.

At present we are investigating the possibility of fractionating nuclei by various mechanical and chemical means with the hope that such procedures may be of assistance in elucidating the role of the nucleus in the synthesis of protein. We also plan to study the proteins of the non-sedimentable fraction at the time when the liberation of protein from the nucleus appears to be taking place.

REFERENCES


EFFECTS OF X RAY ON FERTILITY IN THE MOUSE

M. Sanderson and S. P. Stearner

Monthly breeding trials of X-irradiated, female BA F₁ mice have been continued for nine months following exposure. Dosages ranged from 50 r to 600 r total body X ray and 15-45 mice were included at each dose level. Only two animals have produced viable offspring. One received 100 r and produced one litter in six matings (at the first trial), and one received 200 r and produced five litters in eight matings.

Control females have produced litters consistently. Mean litter size from the first mating was 7.2 offspring. This number increased with subsequent mating, until the mean size at the sixth trial was 9.7. The mean appeared to decrease somewhat at the eighth mating, but the number of animals in the group is as yet small.

Female LA F₁ mice have received X ray doses ranging from 10 r to 100 r at a lower exposure rate, and are now being mated for the first time.
EFFECT OF TEMPERATURE ON PHOSPHATE RELEASE

John M. Ginski

As described in a previous report (ANL-5332) the effect of temperature on phosphate release from the dog erythrocyte is being studied. After dog blood is incubated with radioactive phosphate ($^{32}$P), the erythrocytes are suspended in non-radioactive plasma and the rate of radioactive phosphate release is measured.

Since the last quarterly report, the phosphate release study has been extended to include six temperatures: 27, 30, 32, 35, 37 and 42°C. The rate of phosphate release increases with temperature, with a positive temperature coefficient and an apparent activation energy (calculated according to the Arrhenius equation) of over 50,000 calories per mole.

The $^{32}$P solution (Oak Ridge) used was previously hydrolyzed and oxidized by nitric acid and further oxidized by 30% hydrogen peroxide. This treatment was undertaken to convert the $^{32}$P to the orthophosphate form. When this solution was subjected to paper electrophoretic separation, a subsequent radioautograph revealed more than one distinct radioactive zone. It was apparent that this pre-treatment of $^{32}$P solution did not completely remove all polyphosphates. Thus the $^{32}$P solution used in these experiments was contaminated, at best, with 1 or 2% of polyphosphate.

Steps were taken to obtain $^{32}$P without the impurities of polyphosphate. This was accomplished by the method of Sato, Kisieleski, Norris, and Strain. Experiments were performed with this purified $^{32}$P solution at two temperatures, 27 and 35°C. When the results of these experiments with the contaminated and purified solutions were compared, it was found that, with the purified $^{32}$P solution, the amount of phosphate released at the lower temperature was markedly increased; there was a less marked increase at the higher temperature.

When the temperature coefficients were calculated for the experiments using purified $^{32}$P, values of 0.03 for 35°C and 0.02 for 27°C were obtained, whereas the temperature coefficients calculated for the experiments using the $^{32}$P contaminated with polyphosphate yielded values of 0.03 for 35°C and 0.004 for 27°C. The value obtained for apparent activation energy is dependent upon the difference in temperature coefficients. Consequently, in the experiments in which purified $^{32}$P was used, the apparent activation energy is substantially decreased.

Since different results were obtained using purified $^{32}$P solution and polyphosphate-contaminated $^{32}$P solution, greater care must be taken to insure the homogeneity of radioactive phosphate.
REFERENCE

THE KINETICS OF GLYCINE INCORPORATION BY ESCHERICHIA COLI DURING EXPONENTIAL GROWTH

A. L. Koch

During a study of protein turnover in growing bacteria it became necessary to have detailed information on the kinetics of the uptake of 2-C\(^14\)-glycine by growing cultures. The kinetics were followed by the expedient of determining the decrease in radioactivity of the supernatant solution after high speed centrifugation or by measuring the activity of the well-washed pellet.

If it is imagined that the uptake is dependent on some enzymes that are present in a constant amount in a bacterium, then it follows that the amount of enzyme present in the culture per unit volume is exponentially increasing. This of course renders the usual formulations of enzymology inapplicable. They can be applied, however, in a modified form.

If we let \( S \) be the substrate concentration at any time, \( F(S) \) be the rate of utilization of the substrate per bacterium, and \( k \) be the growth constant; then in a culture, the bacterial titer of which is \( N \), the rate of utilization of the substrate will be given by \(-dS/dt = F(S) N \) and the growth rate by \( dN/dt = kN \). Dividing these two equations, we obtain \(-dS/dN = F(S)/k \). Thus the slope of the plot of \( S \) versus \( N \) yields \( 1/k \) times the utilization rate. The quantity \( F(S)/k \) is of interest in its own right, since it is the amount of glycine at a given concentration that is utilized for the synthesis of one bacterium.

A number of vials containing synthetic glucose medium were inoculated with a culture of actively growing Escherichia coli strain B/1,5. All the vials contained a constant amount of isotope but increasing amounts of carrier glycine. The vials were shaken at 37°C for four hours, during which time the number of bacteria in each vial had increased 31 times. Note that glycine is in no wise an essential metabolite for this organism and does not influence the growth rate. The cells were harvested, washed, plated and counted. The logarithm of the fraction of isotope taken up by the bacteria gives a linear plot against the logarithm of the initial glycine concentration, over the range in which the glycine concentration does not change appreciably during the experiment. The least-squares regression line is given by:

\[ \log R = 1.324 - 0.79 \log S_0, \]

where \( R \) is the fraction of isotope appearing in the bacteria, and \( S_0 \) is the initial concentration in \( \mu M \) per liter. From this it is apparent that the quantity \( F(S) \) depends on the \((1 - 0.79)\), or 0.21, power of the substrate concentration over several orders of magnitude. In Figure II-31 the quantities \( F(S) \) and \( F(S)/k \) are plotted as a function of the logarithm of substrate concentration.
At low concentrations of glycine this method is inapplicable; therefore, experiments were designed to measure the disappearance of substrate in the supernatant as a function of bacterial numbers. It was found that the slopes of such graphs were constant and could be expressed by the integral of the basic equation, assuming \( F(S) \) is a constant, \( V \). One obtains

\[
S = S_0 - V(N - N_0)/k.
\]

The results of two typical experiments are shown in Figure II-32. In the upper experiment, the amount of glycine that is utilized for the synthesis of one bacterium \( (F(S)/k) \) is \( 1.57 \times 10^{-16} \) moles. In the lower experiment, purines were present in the synthetic medium so that the bacteria failed to utilize the glycine for the synthesis of bacterial purines, using, instead, the exogenous purine. In this case \( F(S)/k \) was reduced to \( 0.76 \times 10^{-16} \) moles per bacterium (for the synthesis of bacterial protein). These results are in accord with the results of other experiments done under the same conditions, in which the experiment was stopped at a time when the glycine was still being utilized at a constant rate; the cells were harvested, partitioned into protein and nucleic acid fractions, and the activity associated with the new cells determined. The quantity \( F(S)/k \) determined in this latter way is \( 1.76 \times 10^{-16} \) moles per bacterium, and for the synthesis of bacterial protein is \( 0.85 \times 10^{-16} \) moles per bacterium.

It will be noted that all of the activity is not removed by the bacteria. Other experiments indicate that this activity is associated with compounds that the bacteria produce during metabolism of the radioglycine; these compounds diffuse into the medium and are utilized at a much slower rate than is free glycine, actually at a rate only 0.5 per cent as fast as is free glycine. When the \( ^{14}C \) is reutilized it is distributed into the bacterial proteins and purines in the same fashion as is glycine. Since this material is readily dialyzable it is thought that it is probably a peptide or a mixture of peptides of glycine. It should be noted that the same phenomenon is observed when beta-labeled serine is the precursor.

Another point to be made from these experiments is that the utilization of glycine occurs without a lag, immediately after the addition of glycine to the medium. This is of interest because the organisms had previously been grown in the absence of glycine. The immediate utilization also occurs if the glycine concentration is very high.
Figure II-32 Upper Experiment. The initial titer was $1.6 \times 10^8$ bacteria per ml, the growth rate constant was 0.75 per hour throughout the 2 hours and 50 minutes of the experiment.

Lower Experiment. The initial titer was $2.6 \times 10^8$ bacteria per ml. The growth constant was 0.70 per hour during the 2 hours and 30 minutes the experiment was conducted. Adenine and guanine were present in the subinoculum and the growth flask in a concentration of 2160 &mu;M and 210 &mu;M per liter, respectively.
A COMPARISON OF THE RATE OF UPTAKE OF \textsuperscript{2-\text{C}}\textsuperscript{14}-GLYCINE
BY BACTERIA IN VARIOUS PHASES OF GROWTH

A. L. Koch

As noted in the accompanying communication, glycine is utilized when present in small concentrations at a rate that is independent of glycine concentration. It is of interest to compare the rates of utilization in the case where cells are actively multiplying with other phases of the bacterial growth cycle. We have therefore performed similar experiments with washed cells from actively growing broth cultures. Cells from broth cultures are much larger than those grown in the synthetic medium, and there is a considerable lag phase before they start to grow in the synthetic medium. However, the plating efficiency of broth-grown organisms is the same as synthetic and broth plates, thus, we may conclude that essentially all cells are capable of growth and that the lag phase is characteristic of all cells in the population (G. Sacher and G. Wendorf, personal communication). In the upper portion of Figure II-33 is plotted the amount of substrate remaining in the medium as a function of time for a number of experiments with different initial inocula of broth-grown bacteria. The number of cells as determined turbidimetrically in each culture is indicated in the bottom portion of the graph. Since the calibration curve was standardized with cells grown in synthetic medium, the actual bacterial concentration is considerably smaller during the lag phase than is indicated here. However, the curve is very closely representative of the amount of bacterial protoplasm present at any time.

The rate of uptake per unit of bacterial protoplasm may be computed by dividing the rate of uptake per unit time by the apparent number of organisms present during the lag phase. As can be seen from Figure II-33, the bacteria do not immediately grow at an exponential rate upon leaving the lag phase. However, here the rate of utilization may be computed in a manner similar to that used in the case of exponentially growing cultures. These results are summarized in Table II-20. It can be seen that, per unit protoplasm, lag-phase cells are incorporating glycine at a somewhat smaller rate than are exponentially growing cells, with the intermediate phase (end of lag, beginning of exponential) having a rate between these values. If the results are calculated on the basis of actual bacterial numbers, the lag-phase cells take up glycine at somewhat higher rate than do the exponentially growing cells.
Figure II-33  In all experiments actively growing cells in broth culture were washed and inoculated at various concentrations into synthetic medium containing $2\cdot{\text{C}}^{14}$-glycine. The upper graph shows the amount of glycine activity remaining in the supernatant solutions as a function of time. The lower graph shows the growth of bacteria as determined turbidimetrically with a calibration curve standardized for bacteria growing in the synthetic medium. Thus the actual initial titer is 1.8 times larger than indicated.
TABLE II-20

The rate of uptake of 2-C\textsuperscript{14}-glycine by cultures of *Escherichia coli* in various phases of growth\textsuperscript{a}

<table>
<thead>
<tr>
<th>Phase</th>
<th>Rate of uptake (V)  (10^{-16}) moles per unit of bacterial protoplasm per hour\textsuperscript{b}</th>
</tr>
</thead>
</table>
| Lag Phase\textsuperscript{c} | 0.79  
                                  | 0.73  
                                  | 0.64  |
| Early Growth\textsuperscript{d} | 0.92  
                                  | 1.02  
                                  | 0.86  
                                  | 1.10  |
| Exponential Growth\textsuperscript{e} | 1.18  
                                  | 1.14  |

\textsuperscript{a}The initial concentration of glycine was 100 \(\mu\)M per liter in all experiments except those for exponential growth where the concentrations were 128 and 46 \(\mu\)M per liter, respectively.

\textsuperscript{b}The unit amount of bacterial protoplasm is that associated with one actively growing cell in the synthetic glucose medium. To convert the lag phase data to the basis of bacterial numbers the results should be multiplied by 1.8.

\textsuperscript{c}The rate of uptake, \(V\), is measured as the slope of the graph of concentration of glycine in the medium versus time divided by the apparent bacterial titer.

\textsuperscript{d}The values of \(V/k\) and \(k\) are changing during this phase. The product of the graphical average of these two quantities is tabulated.

\textsuperscript{e}See the accompanying paper.
Distribution studies of the activity in the various bacterial fractions indicate a very similar picture for the lag-and exponential-phase cells (Table II-21). It can therefore be concluded that these cells show a very different metabolic behavior than has been found by Morse and Carter\(^1\) for cells taken from the stationary phase in broth and introduced into synthetic medium. They found that the majority of isotope (P\(^{32}\)) entered the RNA fraction and that RNA synthesis is particularly prominent during lag phase. Thus it would appear that the cells studied here, while adapting to grow on glucose instead of the complex broth medium, are continuously laying down proteins and nucleic acids of both types, but do so in a manner such that the rate of uptake of glycine is maintained constant and the turbidity of the culture is maintained constant during the lag phase.

**TABLE II-21**

<table>
<thead>
<tr>
<th></th>
<th>Lag Growth Phase(\dagger)</th>
<th>Exponential Growth Phase(\dagger)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(10^{-16}) Moles glycine per unit bacterial protoplasm per hour(\ast)</td>
<td>(10^{-16}) Moles glycine per unit bacterial protoplasm per hour</td>
</tr>
<tr>
<td>5% Trichloracetic Acid-Soluble</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>RNA</td>
<td>0.29</td>
<td>0.42</td>
</tr>
<tr>
<td>DNA</td>
<td>0.11</td>
<td>0.13</td>
</tr>
<tr>
<td>Protein</td>
<td>0.30</td>
<td>0.57</td>
</tr>
<tr>
<td>Total</td>
<td>0.79</td>
<td>1.18</td>
</tr>
</tbody>
</table>

\(\dagger\)Actively growing cells in broth were harvested, washed, and suspended in glucose M-9 medium containing 100 \(\mu\)M glycine per liter, initial titer \(5.1 \times 10^6/\text{ml}\). The cells were harvested at the end of 35 minutes. The titer was then \(5.2 \times 10^6/\text{ml}\) and 23 per cent of the glycine was incorporated by the bacteria. The tabulated results were calculated by dividing the \(\mu\)M of glycine (as determined by the radioactivity assay) by the number of bacteria analyzed and by the duration of the experiment.

\(\dagger\)To actively growing cells in glucose M-9 medium was added 2-\(\text{C}^{14}\)-glycine to make 100 \(\mu\)M per liter. In the ensuing 100 minutes the titer rose from 2.1 to \(7.1 \times 10^6/\text{ml}\). Eighty-one per cent of the radioactivity was incorporated by the bacteria. The tabulated results were computed by dividing the \(\mu\)M of glycine by the increment of bacterial numbers and then multiplying by the growth rate constant (0.68 per hour).

\(\ast\)See footnote b of Table II-20.
REFERENCE

APPLICATION OF LEUCOCYTE ANTISERUM TO STUDY OF RADIATION-INDUCED LEUCOPENIA

H. M. Patt, M. A. Maloney, and E. M. Jackson

It was noted previously that the capacity for peripheral neutrophil recovery to the level existing before a single injection of leucocyte antiserum (rabbit antidog) decreased progressively with time after total body, median lethal, X-irradiation (ANL-5288, p. 40). The data were consistent with the idea that recovery occurred mainly at the expense of a reservoir of relatively advanced neutrophil precursors which became depleted with time after irradiation. The present experiments were undertaken to determine the effect of an abrupt decrease in circulating white blood cells resulting from repeated injections of antiserum during the first day after exposure on the subsequent radiation picture. It was reasoned that severe leucopenia induced in this way might perhaps activate primitive precursor cells that were still capable of responding to a physiological signal to blood formation; it was recognized, however, that this procedure might also enhance radiation injury by imposing an additional stress on the system. Results obtained in a small series of dogs indicate that antiserum injections during the first hours after X-irradiation with 300 r appear to diminish the magnitude and duration of radiation leucopenia and, concomitantly, to favor survival (6 of 8 treated survivors at 30 days as compared with 2 of 8 irradiated controls). Similar treatment after exposure to 400 r has been comparatively ineffectual in the few dogs that have been studied. Further experiments are in progress to evaluate these findings more completely.
Leucocyte recovery in the dog after acute peripheral depletion appears to be qualitatively similar whether the reduction in white blood cells is achieved by exchange transfusion with leucocyte-free blood (ANL-5200, p. 10) or by injection of leucocyte antiserum (Figure II-34). It is suggested that the mechanism of compensation for the decline in cells produced by either technique may involve a physiological negative feedback between circulating leucocytes and bone marrow. For convenience, leucocyte antisera (rabbit antidog) have been used most extensively in these studies. Although the relationship between the degree of leucopenia and the intensity of marrow stimulation has not yet been fully characterized, there is some indication of a linear increase in nonsegmented neutrophils during the recovery period as the minimal neutrophil level decreases exponentially. When neutropenia is maintained for several hours by repeated or continuous injection of antiserum, there is a progressive shift in the direction of greater immaturity, with the appearance of significant numbers of metamyelocytes and myelocytes in the peripheral blood. It may be noted that the pattern of leucocyte recovery is similar in normal and splenectomized dogs. Present experiments are concerned more directly with the nature of the mechanisms that may be involved in regulation of neutrophil levels and include parallel observations of hematopoietic tissue and peripheral blood.
Figure II-34  Segmented and nonsegmented neutrophils in peripheral blood after exchange transfusion with leucocyte-free blood (1500 ml. in 20 minutes) or I.V. injection of leucocyte antiserum (0.5 ml./kg. B.W. 1:10 dilution).
THE USE OF PLASTICS IN THE PREPARATION OF THIN SECTIONS OF UNDECALCIFIED BONE; WITH SPECIAL REFERENCE TO RADIOAUTOGRAPHIC PROCEDURES

Lois A. Woodruff and William P. Norris

A rapid technique for the preparation of 6μm sections of undecalcified bone has been devised. The method requires a minimum of time (one week to produce a block ready for sectioning), is reproducible, and is applicable to human bone as well as bone of laboratory animals.

Long bones of small animals are cut in half longitudinally and areas of larger bones are selected. The tissue is fixed and dehydrated in absolute acetone and defatted in diethyl ether. Further defatting is accomplished in a 1:1 mixture of the monomers n-butyl methacrylate and ethyl methacrylate (minus inhibitor) catalyzed with 0.05% 2,4-dichlorobenzoyl peroxide. The monomers are removed with ether and the ether is pumped off under vacuum.

A partial polymer is prepared by heating the same 1:1 mixture of monomers at ~65°C until a syrup is formed. The tissue is infiltrated with the syrup under vacuum and then transferred to a square glass bottle. The specimen is oriented with the surface to be cut resting on a plastic plug previously polymerized in the bottom of the bottle. The bottle is 3/4 filled with syrup and polymerization is completed in a pressurized vessel at 50 psi (tank nitrogen) and at 50°C.

In 24-36 hours, the mold is broken off and the excess plastic surrounding the specimen sawed away. The plastic block is inserted directly into the vise of a heavy-duty microtome. The knife and block are wet with 70% alcohol during sectioning and the sections are mounted on "subbed" slides. The plastic is removed by immersion in acetone. The sections now can either be stained and examined histologically or coated with stripping film emulsions for radioautographic studies.

Structural detail is preserved and there has been no evidence of translocation of radioactivity in work with Ca⁴⁵, Sr⁹⁰, and Ra and Pu²³⁹. Sections prepared in this way should be useful also in microradiographic and histochemical studies on bone.
Figure II-35  A low power photomicrograph of a longitudinal section (6 μ) through the proximal end of the tibia of a young pig.

Figure II-36  The epiphyseal plate and primary spongiosa from a longitudinal section (6 μ) of the distal end of a dog femur.
Figure II-37 A longitudinal section (6μ) through the cortex of a normal adult rat.

Figure II-38 Radioautograph of a longitudinal section (6μ) through the femoral cortex of an adult rabbit. This animal was sacrificed 15 days after receiving 75 μg Pu$^{239}$. Alpha tracks demonstrate the concentration of Pu above the endosteal surface. There is a lesser concentration along the periosteum.

Figure II-39 Radioautograph of a section (6μ) cut longitudinally through the distal end of a rabbit femur. The rabbit received 75 μg Pu$^{239}$ 15 days prior to sacrifice. Alpha tracks demonstrate the localization of Pu near the surface in trabecular bone.
PRELIMINARY EXPERIMENTS ON THE MECHANISM OF
SPLEEN PROTECTION

H. S. Ducoff

It has been suggested by various workers that the X-ray protection
factor in mouse spleen is a subcellular material promoting earlier regen-
eration of radiation-damaged hemopoietic and allied systems. Assuming
this to be the case, elucidation of the mechanism of action of the factor
depends on three lines of investigation:

1. The chemical nature of the factor
2. The nature of the lesion against which the factor is effective
3. The necessity for the presence of cellular systems, metabolites,
etc., as substrates or cofactors for the action of the splenic
factor.

The CF #1 female mouse has been employed as the experimental
animal in the present work. In the first few experiments, mice received
at 3-4 weeks of age served as donors. Irradiated groups injected with
homogenates of spleens from these mice often died within four or five days;
blood cultures* of these animals revealed the presence of Salmonella
enteritidis. In all subsequent experiments, except where otherwise noted,
donors were two-week-old mice bred in the Laboratory.

Homogenates were prepared in ATP-fortified buffered sucrose(1)
centrifuged in the cold, and the residue resuspended in fresh medium so
that one milliliter of washed homogenate was equivalent to 100 mg spleen.
One-half ml of homogenate, intraperitoneally, was the usual dose. When
the washing was omitted, toxic reactions in the recipient mice were often
noted, with a relatively large proportion of early deaths.

In an experiment to secure further information on the chemical
nature of the factor, homogenates were exposed in vitro to a nitrogen
mustard for 30 minutes (8 μg HN-2/cc) and to acriflavin (6 μg/cc) for
60 minutes. No survivors were found at 21 days among eight mice ex-
posed to 725 r and then injected with the nitrogen mustard-treated homog-
enate. Four of 11 receiving acriflavin-treated, washed, homogenate and
7 of 11 receiving untreated, washed, homogenate survived for more than
30 days. Of 10 mice exposed to 725 r and otherwise untreated, none survived
for 21 days. It seems fairly certain that the acriflavin did not completely
inactivate the protective factor, but with such small numbers it is not
possible to estimate how much if any inactivation occurred.

* Diagnosis was done with the collaboration of George Collins.
TABLE II-22
PROPORTION OF SPLEEN-TREATED ANIMALS SURVIVING 21 DAYS
AFTER EXPOSURE TO 740 r

<table>
<thead>
<tr>
<th>Spleen preparation injected</th>
<th>Intact</th>
<th>Splenectomized</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-week-old donors, injected 2 hours after exposure</td>
<td>3/7</td>
<td>0/9</td>
<td>3/16</td>
</tr>
<tr>
<td>Adult donors 14 days after 400 r, injected 20 hours after exposure</td>
<td>3/10</td>
<td>2/9</td>
<td>5/19</td>
</tr>
<tr>
<td>2-week-old donors, injected 20 hours after exposure</td>
<td>2/4</td>
<td>2/7</td>
<td>4/11</td>
</tr>
<tr>
<td>Total</td>
<td>8/21</td>
<td>4/25</td>
<td>12/46</td>
</tr>
</tbody>
</table>

The size of these experiments is severely limited by the small amount of spleen obtainable from each baby mouse (less than 10 mg) and the number of spleens which can be harvested within a reasonable time. Since spleens of adult mice recovering after X irradiation exhibit foci of mitotic activity, ectopic hematopoiesis, and a transient hypertrophy, such spleens might prove a source of the protective factor. It was also of interest to determine whether an irradiated mouse could respond to the splenic factor in the absence of its own spleen, or whether there exists some form of "substrate specificity." Thirty-one intact animals, and 25 which had been splenectomized† 14 days earlier, were exposed to 740 r. Ten of the intact mice received no further treatment, and all were dead by the fifteenth post-irradiation day. The remaining irradiated animals were injected within 2 hours with a homogenate of two-week-old spleens, or were injected 20 hours later with homogenate of either spleens of adult mice exposed to 400 r 14 days earlier, or of spleens of two-week-old mice. The data on 21-day survival are shown in the table. Again, comparison between different treatment groups is unreliable because of the small numbers involved. It is clear from the totals, however, that each preparation exerted some protective activity, and that the splenectomized animal can still be protected by spleen homogenate after otherwise lethal irradiation.

†Splenectomies were performed by Agnes N. Stroud.
These observations are being extended by estimation of the protective activity of adult spleens at various times after 400 r, and by experiments on folic acid-deficient mice and on animals with bone marrow damaged by aminopterin.

REFERENCE

THE EFFECT OF SERUM, PLASMA PROTEINS, AND OTHER SUBSTANCES ON THE SURVIVAL OF ASCITES TUMOR CELLS AFTER X-IRRADIATION

Agnes N. Stroud and Austin M. Brues

In view of our interest in the protective action of plasma, serum, and plasma proteins when given to mice before irradiation, it seemed worthwhile to investigate this action on a cellular level, since it is not yet clear whether this protection acts through some gross physiological action on the whole animal or on a more general cellular basis.

The Krebs 2 ascites tumor was used as the test-object since it is a rapidly growing radiosensitive neoplasm and well adapted to quantitative observations. The ascitic fluid from a mouse inoculated 8 days previously was mixed with an equal volume of physiological saline or test substance and irradiated with 2000 r at a rate of 35 r/min. Two separate experiments were done under conditions to ensure that the radiation doses to all of the suspensions were equal. Mice were then injected intraperitoneally with irradiated or control suspensions containing approximately $5 \times 10^6$ cells. The mice were weighed daily and autopsied to verify the presence of tumors. The results are shown in Table II-23. All mice inoculated with unirradiated cells were dead by the 22nd day. It is noted that 2000 r is not completely lethal for the tumor, since one animal injected with a suspension irradiated in saline died on the 26th day. The results strongly suggest that fraction III exerts some protective action on the tumor cells but that the resulting ascitic tumors are delayed in their appearance. Verification of these results is being undertaken.

Suspensions of cells irradiated with 1000 r, in the presence of the same test substances, did not differ measurably in their viability from unirradiated suspensions.
### TABLE II-23

<table>
<thead>
<tr>
<th>X ray dose to cells</th>
<th>Exp. group</th>
<th>Substance</th>
<th>% dead of tumor by 50 days</th>
<th>mean date of death from tumor (days)</th>
<th>No. of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>I</td>
<td>Isotonic NaCl</td>
<td>100</td>
<td>14.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Albumin</td>
<td>100</td>
<td>14.7</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse serum</td>
<td>100</td>
<td>13.7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fraction III* (.3 mg)</td>
<td>100</td>
<td>19.8</td>
<td>10</td>
</tr>
<tr>
<td>2000 r</td>
<td>II</td>
<td>Isotonic NaCl</td>
<td>100</td>
<td>14.5</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sub-fraction III*</td>
<td>100</td>
<td>14.5</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>Isotonic NaCl (larger cell inoculum)</td>
<td>100</td>
<td>11.5</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>Albumin</td>
<td>0</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse serum</td>
<td>0</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse serum (diluted)</td>
<td>0</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fraction III (.3 mg)</td>
<td>0</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fraction III (.15 mg)</td>
<td>20</td>
<td>30.5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fraction III, saturated</td>
<td>30</td>
<td>41.5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>Isotonic NaCl</td>
<td>7</td>
<td>26.0</td>
<td>15</td>
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<td></td>
<td></td>
<td>Sub-fraction III*</td>
<td>60</td>
<td>20.1</td>
<td>15</td>
</tr>
</tbody>
</table>

* Blood protein fraction (human)
THE EFFECT OF FLUOROACETATE ADMINISTRATION ON
EXPERIMENTAL PLUMBISM

Joan F. Fried and Marcia White Rosenthal

Studies are being resumed on the relationship of fluoroacetate ad­
ministration to experimental lead poisoning. Previous experiments in this
laboratory have demonstrated that administration of fluoroacetate in sub­
lethal doses to rats has resulted in marked increase in citrate content of
the tissues. Elsewhere, citrate administration has been shown to be of
therapeutic value in some cases of human plumbism, probably because of
the ability of the citrate ion to form a soluble complex ion with the lead in
the tissues and circulatory system. These facts suggest that stimulation
of citrate production in vivo by means of fluoroacetate administration may
afford protection to experimental plumbism, and survival studies have ac­
cordingly been conducted.

Pb(NO₃)₂ was administered intravenously to Sprague-Dawley female
rats at a level of 70 mg/kg; this dose resulted in 6% survival of the saline­
treated control groups (50 animals). Sodium fluoroacetate was administered
by intraperitoneal injection both before and after Pb(NO₃)₂, injection to the
experimental groups (115 animals) at several levels ranging from 0.24 to
0.48 mg/kg, and at varying time intervals. To date, the most significant
protection (40-80% survival within 14 days) has been achieved by giving an
injection of 0.30 mg/kg twice before the Pb(NO₃)₂ and twice daily for 1–
3 days afterwards. In addition, the survival time of the animals that died
has been prolonged 2-4-fold. Survival studies are being continued in an
effort to establish conclusively the most effective schedule of treatments,
which will presumably reflect the maintenance of optimal citrate levels
in the tissues.
STATISTICAL ANALYSIS OF NEUTROPHIL, LYMPHOCYTE AND ERYTHROCYTE COUNTS FROM A NORMAL HUMAN POPULATION

J. M. Gurian and G. A. Sacher

In a previous study the mean values and variability of neutrophil and lymphocyte counts and their correlation were presented for three individuals on whom blood counts were taken almost daily for periods up to two years (ANL-4360, p. 43). The present study presents the same statistics for a population of project workers, for each of whom only a few determinations are available, and includes also the parameters for the erythrocyte counts. The purpose of these studies is to describe the blood picture of a normal human population and the individuals comprising it, the interdependence of the blood variables, and their dependence on age or sex.

The population consists of white individuals of both sexes, ranging in age from 16 to 75 years. It was divided into groups by sex and decade of age. The data being used are the blood counts routinely obtained at the Metallurgical Laboratory during 1943-5. The values of the neutrophil and of the lymphocyte counts were calculated from the white cell count and the differential counts made at the same time.

For each group the mean and the standard deviation of the logarithm of the neutrophil count, of the logarithm of the lymphocyte count, and of the absolute value of the erythrocyte count were determined. The logarithms of the neutrophil and the lymphocyte counts were used because this transformation gives a distribution more nearly normal than the absolute values. In Table II-24 are presented the geometric mean (the antilogarithm of the arithmetic mean of the logarithms of the variables) and the coefficient of variation (C.V.) of the neutrophil and the lymphocyte counts, and the arithmetic mean and the standard deviation (σ) of the erythrocyte counts. The coefficient of variation is defined as one hundred times the excess beyond one of the antilogarithm of the standard deviation of the logarithms of the variables. The geometric mean is smaller than the arithmetic mean and closer to the median of the population. The sample of erythrocyte counts is smaller than that of neutrophil and lymphocyte counts, because the white counts were usually made at three-month intervals, while the red blood cell counts were made at six-month intervals.

There appears to be no trend with age either for the means or the coefficient of variation, except possibly for neutrophils and lymphocytes at the highest ages, where, however, the sample is too small to give well defined results.
TABLE II-24

Means, Dispersion of Neutrophil, Lymphocyte and Erythrocyte Counts

| Age group | Neutrophils | | | Lymphocytes | | | Erythrocytes | | |
|-----------|-------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|           | No. of counts | Mean (×10⁶) | C.V. (%) | Mean (×10⁶) | C.V. (%) | Mean | N | Mean (×10⁶) | C.V. (%) | N | Mean (×10⁶) | C.V. (%) | σ |
| Males:    |             |               |          |               |          |      |    |               |          |    |               |          |   |
| 16-19     | 176         | 4009          | 34.8    | 2470          | 34.5    | 87   | 48.47 | 4.12 |
| 20-29     | 1761        | 3786          | 35.8    | 2393          | 33.2    | 299  | 48.71 | 5.14 |
| 30-39     | 675         | 4147          | 36.0    | 2453          | 34.5    | 381  | 47.88 | 4.23 |
| 40-49     | 389         | 4409          | 35.7    | 2489          | 34.7    | 237  | 46.99 | 4.31 |
| 50-59     | 364         | 4600          | 35.5    | 2492          | 37.3    | 237  | 46.30 | 3.82 |
| 60-69     | 107         | 4288          | 26.9    | 2217          | 32.7    | 61   | 46.33 | 6.19 |
| 70-75     | 15          | 4279          | 17.8    | 2106          | 30.9    | 12   | 44.83 | 3.63 |
| Females:  |             |               |          |               |          |      |    |               |          |    |               |          |   |
| 16-19     | 83          | 4451          | 38.1    | 2324          | 34.1    | 59   | 44.27 | 4.68 |
| 20-29     | 575         | 4221          | 37.1    | 2362          | 32.0    | 380  | 42.50 | 3.48 |
| 30-39     | 129         | 4303          | 37.1    | 2333          | 31.0    | 87   | 43.04 | 3.96 |
| 40-49     | 90          | 4530          | 39.0    | 2380          | 35.6    | 60   | 43.45 | 4.36 |
| 50-59     | 28          | 3869          | 26.1    | 2144          | 26.0    | 25   | 44.04 | 3.54 |
| 60-69     | 5           | 4475          | 17.6    | 1814          | 25.2    | 3    | 47.00 | 5.29 |

Coefficients of correlation of each variable with each of the others were calculated for each group, again based on the logarithm of the neutrophil count, the logarithm of the lymphocyte count, and the absolute values of the erythrocyte count. These are presented in Table II-25. The number of counts is smaller than in Table II-24, since data from individuals for whom at least two counts are given were used. From the third through the sixth decade the correlation between any two of the investigated variables appears to be positive.

Additional calculations, aiming at more detailed biometric description of the blood picture of a normal human population, are in progress.
### TABLE II-25

Correlation Coefficients, Neutrophil-Lymphocyte, Neutrophil-Erythrocyte and Lymphocyte-Erythrocyte

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of counts</th>
<th>$r_{NL}$</th>
<th>No. of counts</th>
<th>$r_{NE}$</th>
<th>No. of counts</th>
<th>$r_{LE}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-19</td>
<td>176</td>
<td>.2470</td>
<td>72</td>
<td>-.0526</td>
<td>72</td>
<td>-.0174</td>
</tr>
<tr>
<td>20-29</td>
<td>1758</td>
<td>.1584</td>
<td>657</td>
<td>.0704</td>
<td>657</td>
<td>-.0403</td>
</tr>
<tr>
<td>30-39</td>
<td>674</td>
<td>.2814</td>
<td>329</td>
<td>.0105</td>
<td>329</td>
<td>.1172</td>
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<td>40-49</td>
<td>389</td>
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<td>215</td>
<td>.1102</td>
<td>215</td>
<td>.0544</td>
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<td>50-59</td>
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<td>.3269</td>
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<td>60-69</td>
<td>107</td>
<td>.1595</td>
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<td>.0483</td>
<td>49</td>
<td>.2287</td>
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<td>.5776</td>
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<td>-.0432</td>
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<td>.3486</td>
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<td>Females:</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>16-19</td>
<td>82</td>
<td>-.0797</td>
<td>51</td>
<td>.0513</td>
<td>51</td>
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<td>.1455</td>
<td>332</td>
<td>.1179</td>
<td>332</td>
<td>.1080</td>
</tr>
<tr>
<td>30-39</td>
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<td>.0135</td>
<td>72</td>
<td>.0587</td>
<td>72</td>
<td>.1458</td>
</tr>
<tr>
<td>40-49</td>
<td>88</td>
<td>.2956</td>
<td>43</td>
<td>.2521</td>
<td>43</td>
<td>.2183</td>
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<td>28</td>
<td>.1999</td>
<td>25</td>
<td>.2145</td>
<td>25</td>
<td>-.1032</td>
</tr>
</tbody>
</table>
MURINE MANGE CONTROL PROGRAM

R. J. Flynn and B. N. Jaroslow

Murine mange is a problem in all laboratories where mice are kept for lifetime studies and because our work involves such studies, a program of control would be desirable.

Three species of mites have been observed and identified on the mice at Argonne National Laboratory. Two of these mites have been reported frequently in the literature, namely, Myobia musculi, and Myocoptes musculinus. The other, a rarely reported ectoparasite of mice, has been named Psorergates simplex by Tyrrell (1,2) and Goniomercus musculinus by Michael (3). Baker (4) of the U.S.D.A. (National Museum) suggests the use of the name Psorergates simplex.

Myobia musculi (Figure II-40) was first described by Schrank (5) in 1781. The life cycle and habits of this acarinid, particularly whether or not it invades the hair follicles to feed and moult, has been debated for some years (6, 7, 8, 9). The most complete and probably correct report is that of Grant (9). He fully described the nymphal and larval stages, but did not present any data on the time required for completion of each successive stage and for the entire cycle. The incidence of infestation of M. musculi at this laboratory, based upon the careful examination of approximately 40 CF#1 mice ranging in age from 1 to 8 months, was found to be 100%.

Myocoptes musculinus (Figure II-41) was first described by Koch (10) in 1836. Little has been done on the morphology and life cycle of this mite since that time, and the various stages and life cycle have yet to be reported. The incidence of infestation of M. musculinus at Argonne National Laboratory, based upon the careful examination of approximately 30 CF#1 mice ranging in age from 1 to 8 months, was found to be 100%.

Psorergates simplex (Figure II-42) was first described by Tyrrell (1,2) in 1882. Recently, Wassermann of this laboratory injected mice intravenously with trypan blue and, after killing and skinning, noted some spots on the inner surface of the integument where the blue coloring was much less intense than on the rest of the skin (Figure II-43). Fixed in formalin, sectioned and stained, these areas revealed pouches extending through the derma and subcutis to the skin muscle layer (Figure II-44). A smear of the contents of these pouches disclosed Psorergates.

P. ovis has been described by Womersley (11) and reported by Carter (12) as the cause of a mange in sheep in Australia. Bell et al., (13) have reported on P. ovis mange of sheep in Ohio. Carter and Bell both mentioned the difficulty in obtaining positive skin scrapings unless they
Figure II-40  
*Myobia musculi.*

Figure II-41  
*Myocoptes musculinus*

Figure II-42  
*Psorergates simplex*
Figure II-43 Inner surface of integument. Note light areas.

Figure II-44 Section of light area.
were very deep scrapings. If *P. ovis* invades the skin in the same manner that *P. simplex* does, it is obvious why deep scrapings are necessary to demonstrate this acarinid. The difficulty in demonstrating this mite is undoubtedly the reason that its occurrence and incidence in laboratory mice has been overlooked. Using the method of Wassermann, we found that 8 of the 35 CF #1 mice (5 to 8 months of age) examined in our laboratory were infested with this mite.

Whether or not *P. simplex* causes a mange condition in mice as *P. ovis* does in sheep has not yet been determined.

Studies to effectively control mange of laboratory mice have been initiated. For control of the parasitic infestations discussed above, it will be necessary to know the occurrence, habits, and life cycle of the parasites in question.

**REFERENCES**


INDEX

ARGONNE NATIONAL LABORATORY

REPORTS ON BIOLOGICAL, MEDICAL
AND BIOPHYSICS PROGRAMS

ANL-5378    January 1955
ANL-5332    October 1954
ANL-5288    July 1954
ANL-5247    April 1954
<table>
<thead>
<tr>
<th>Term</th>
<th>Page Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acids effect on ribonuclease</td>
<td>5378:133</td>
</tr>
<tr>
<td>Activation analysis of tumor</td>
<td>5247:24</td>
</tr>
<tr>
<td>Activity of beta-ray sources</td>
<td>5288:113</td>
</tr>
<tr>
<td>Adrenal cortex and tissue mast cell in hamster</td>
<td>5378:123</td>
</tr>
<tr>
<td>and tissue mast cell in rat</td>
<td>5332:33</td>
</tr>
<tr>
<td>Age in beryllium toxicity</td>
<td>5247:76</td>
</tr>
<tr>
<td>Air ionization in compared with C₄F₈</td>
<td>5378:5</td>
</tr>
<tr>
<td>Alkali-sensitized positive ion emitters for detection of halogen vapors</td>
<td>5288:131</td>
</tr>
<tr>
<td>Alpha foot monitor</td>
<td>5288:131</td>
</tr>
<tr>
<td>Amino acid biosynthesis</td>
<td>5332:27</td>
</tr>
<tr>
<td>Anhydrous acid effect on protein ultracentrifuge patterns</td>
<td>5288:61</td>
</tr>
<tr>
<td>Antibody formation effect of radiation and protective agents</td>
<td>5247:53</td>
</tr>
<tr>
<td>in x-irradiated rabbits</td>
<td>5288:64</td>
</tr>
<tr>
<td>Antimitotic agent n-ethyl maleimide</td>
<td>5247:43</td>
</tr>
<tr>
<td>Antiserum, leucocyte and leucopenia</td>
<td>5378:167</td>
</tr>
<tr>
<td>Arginase in regenerating liver</td>
<td>5378:150</td>
</tr>
<tr>
<td>Armstrong, C.</td>
<td>5288:79</td>
</tr>
<tr>
<td>Ascaris eggs cleavage time after x-ray</td>
<td>5378:154</td>
</tr>
<tr>
<td>Ascites formation in ascites tumor-bearing mice</td>
<td>5288:46</td>
</tr>
<tr>
<td>Ascites tumor and vascular permeability</td>
<td>5378:71</td>
</tr>
<tr>
<td>growth in hepatectomized mice</td>
<td>5378:71</td>
</tr>
<tr>
<td>radiation effects</td>
<td>5288:7</td>
</tr>
<tr>
<td>Ascites tumor cells protection against irradiation</td>
<td>5378:176</td>
</tr>
<tr>
<td>Ascitic fluid comparison with mouse plasma in ultracentrifuge</td>
<td>5288:60</td>
</tr>
<tr>
<td>Atomospheric diffusion</td>
<td>5378:54</td>
</tr>
<tr>
<td>Auerbach, H.</td>
<td>5332:6</td>
</tr>
<tr>
<td>Aureomycin effect on mouse mortality</td>
<td>5332:18</td>
</tr>
<tr>
<td>Aurintricarboxylic acid (ATA) effect on survival in mice</td>
<td>5288:91</td>
</tr>
<tr>
<td>localization in mouse tissue</td>
<td>5332:90</td>
</tr>
<tr>
<td>therapeutic effectiveness in Be-poisoned mice</td>
<td>5332:92</td>
</tr>
</tbody>
</table>
Aurintricarboxylic acid-aluminum toxicity 5247:79

Aurintricarboxylic acid-iron toxicity 5247:79

Auxin action 5288:58

biosynthesis tryptamine as intermediate 5332:59

Background radiation monitoring system 5378:6

Barton, A. D. 5378:155

Bat (Myotis lucifugus) clotting time and mast cell number 5288:43

Beams, H. W. 5332:41

Berlman, I. B. 5288:114,116; 5378:7

Beryllium effect on enzymatic reaction 5247:78

enzymatic method for analysis 5247:77; 5288:92; 5332:91

localization in mouse tissues 5332:90

poisoning therapeutic effectiveness of ATA in 5332:92

toxicity 5247:76

Beta-radiation ionization around point source 5288:113

Beta-ray sources activity 5288:113

Bink, N. 5247:30

Blackford, M. E. 5247:5; 5288:7

Blood clotting time of bat in different states 5288:43

Blood counts in normal human population 5378:179

Blood-forming tissue reaction to leucocyte depletion 5247:7

Bone phosphate compounds 5247:62

undecalcified thin sections 5378:170

Boron substitutions for in plant growth 5332:54

Bowe, J. C. 5378:40, 48, 50

Brar, S. S. 5288:113,149; 5378:5, 6, 62, 63

Brues, A. M. 5247:8, 23, 24, 25, 61, 80; 5288:23, 85; 5332:5, 79; 5378:72, 100, 102, 106, 176

Buckwheat absorption and utilization of isotopic nitrogen 5288:99; 5332:96

Burch, M. 5247:54
Calcium
mechanism of transport and deposition 5247:65
role of in structure and function of collagenous fibers 5332:47
Calcium$^{45}$ and Ra$^{226}$ to dogs 5378:74
C$_4$F$_8$ gas ionization in 5378:5
Carbon
assimilation in Hevea 5288:100
fixation by E. coli 5247:67
Carbon$^{14}$ infiltration 5288:98
C$^{14}$O$_2$
charger 5288:97
in amino acid biosynthesis 5332:27
Carcinogenicity
of irradiated biological materials 5378:100
Cataract formation
effect of fast neutrons and gamma-rays on 5332:29
Cephalin flocculation test 5247:45
Chamber
gastight 5378:50
Chemostat
mean appearance time of mutants 5288:65
Chick
initial radiation response, role of hypotension 5288:9
radiation protection 5247:72
Chick
survival, effect of dosage rate 5288:38
survival after x-ray and surgery 5378:76
Chilomonas paramecium
effect of ethyl maleimide 5247:43
Chlorella
accumulation of P$^{32}$ by 5332:66
metabolism of 5247:44
Chloroplasts
metabolism 5247:44
Chorney, W. 5288:100; 5332:57
Christian, E. J. B. 5247:8, 72; 5288:9, 38; 5332:13; 5378:76
Chromium$^{51}$ determination 5378:26
Clark, J. W. 5247:30; 5288:35, 37, 39, 83; 5378:118
Clark, R. K. 5288:113,149; 5378:63
Cleavage
of eggs after irradiation 5378:154
Climatological survey 5288:135
Climatology 5378:58
Coincidence circuit for determination of RaTh 5288:127
Collagenous fibers
divisibility of 5332:48
role of calcium in structure and function 5332:47
<table>
<thead>
<tr>
<th>Topic</th>
<th>Page Numbers</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collision cross sections evaluation for metastable atoms</td>
<td>5378:30</td>
<td></td>
</tr>
<tr>
<td>Cooper, D.</td>
<td>5288:37, 83</td>
<td></td>
</tr>
<tr>
<td>Counter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>automatic for micro-organisms</td>
<td>5332:94</td>
<td></td>
</tr>
<tr>
<td>CP-5 reactor modification of radiation chamber</td>
<td>5288:35</td>
<td></td>
</tr>
<tr>
<td>Craig, C. A.</td>
<td>5288:95</td>
<td></td>
</tr>
<tr>
<td>Craig, R. A.</td>
<td>5288:79</td>
<td></td>
</tr>
<tr>
<td>Cysteine protection for antibody-forming mechanism</td>
<td>5247:53</td>
<td></td>
</tr>
<tr>
<td>Death probability in mice after irradiation</td>
<td>5378:118</td>
<td></td>
</tr>
<tr>
<td>Desoxyribonucleic acid stability during growth</td>
<td>5378:148</td>
<td></td>
</tr>
<tr>
<td>Deuterium exchange between hydrocarbons and D₂O</td>
<td>5288:95</td>
<td></td>
</tr>
<tr>
<td>Devine, R. L.</td>
<td>5288:88; 5332:41; 5378:81, 154</td>
<td></td>
</tr>
<tr>
<td>Dogs treated with Ra²²⁶ and Ca⁴⁵</td>
<td>5378:74</td>
<td></td>
</tr>
<tr>
<td>Dosage rate effect on chick survival</td>
<td>5288:38</td>
<td></td>
</tr>
<tr>
<td>Dose-reduction principle failure in E. coli photoreactivation</td>
<td>5288:41</td>
<td></td>
</tr>
<tr>
<td>Dosimetry</td>
<td>5288:113,114,116; 5378:5</td>
<td></td>
</tr>
<tr>
<td>low energy x-ray, phantoms</td>
<td>5378:84</td>
<td></td>
</tr>
<tr>
<td>neutron</td>
<td>5378:7</td>
<td></td>
</tr>
<tr>
<td>Ducoff, H. S.</td>
<td>5247:9, 43; 5288:84; 5378:173</td>
<td></td>
</tr>
<tr>
<td>Ehret, C. F.</td>
<td>5247:55; 5332:75; 5378:152</td>
<td></td>
</tr>
<tr>
<td>Eib, M.</td>
<td>5288:63</td>
<td></td>
</tr>
<tr>
<td>Eisler, W. J., Jr.</td>
<td>5378:6</td>
<td></td>
</tr>
<tr>
<td>Electron microscope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>frog oocytes</td>
<td>5332:78</td>
<td></td>
</tr>
<tr>
<td>improved specimen grid</td>
<td>5332:45</td>
<td></td>
</tr>
<tr>
<td>Malpighian tubules in grasshopper</td>
<td>5332:41</td>
<td></td>
</tr>
<tr>
<td>study of fibril formation</td>
<td>5288:47</td>
<td></td>
</tr>
<tr>
<td>Electronic wind vane</td>
<td>5378:58</td>
<td></td>
</tr>
<tr>
<td>Electrons drift velocity in gases</td>
<td>5378:40</td>
<td></td>
</tr>
<tr>
<td>nor-Epinephrine protection against radiation</td>
<td>5247:72</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>isotope distribution in purines</td>
<td>5378:140</td>
<td></td>
</tr>
<tr>
<td>kinetics of glycine incorporation</td>
<td>5378:159</td>
<td></td>
</tr>
<tr>
<td>photoreactivation</td>
<td>5288:41</td>
<td></td>
</tr>
<tr>
<td>rate of glycine uptake</td>
<td>5378:112</td>
<td></td>
</tr>
</tbody>
</table>
Ethyl maleimide
antimitotic in protozoan cells
5247:43
toxicity for mice 5247:43

5'-Ethylthioadenosine preparation
5378:126

Fast neutron dosimetry 5288:114, 116

Fast neutron irradiation
and streptomycin therapy in
mice 5288:83
and endogenous infection in
mice 5288:37
effect on chick survival 5288:38

Fertility
x-ray effect in mice 5288:66; 5378:156

Fibril
formation in regenerating Achilles
tendon 5288:47
reconstitution in collagen solu-
tions 5288:50

Finkel, M. P. 5247:25; 5288:23; 5332:18; 5378:106

Fission neutrons
spleen weight loss after irradia-
tion with 5288:39

Flavonoids in tobacco 5332:57

Fluoroacetate
in plumbism 5378:178
Flynn, R. J. 5378:182

Formate
fixation of carbon from 5247:67

Free electrons
release of 5378:48

Fried, J. F. 5247:78; 5288:92; 5332:90; 5378:125, 178

Frog oocyte
incorporation of glycine into
5332:52
effect of x-ray on yolk deposi-
tion 5332:53
electron microscopy of 5332:78

Gamma-irradiation
and daily probability of death
in mice 5378:118

Gamma-neutron radiation
chamber, modification of 5288:35

Gamma-ray toxicity program
5378:91

Gamma-rays, effect on
cataract formation in mice
5332:29
chick survival 5288:38
endogenous infection in mice
5288:37
light reflectance in plants 5247:10
spleen weight 5288:39

Gamma rooms 5288:129; 5378:6

Gases
ionization in 5378:30
Gastight chamber 5378:50

Genetic variation
in lethality of irradiated mice 5288:68

Geographic studies in bone tumors 5332:6

Ginski, J. M. 5332:58; 5378:157

Glycine
fixation of carbon from 5247:67

incorporation in frog oocyte 5332:53

kinetics of incorporation by E. coli 5378:159

uptake rate by bacteria 5378:162

Gordon, S. A. 5288:56; 5332:60; 5378:145

Grahn, D. 5247:18; 5288:68; 5332:7; 5378:91

Grape phloem
translocation of C14-labeled compounds 5332:40

Grasshopper embryo
effects of metabolic poisons and oxygen on radiosensitivity 5288:88

irradiation effect on weight and protein 5378:81

Grasshopper
Malpighian tubules 5332:41

Growth and development of soybean induced by radiocarbon 5288:20

Gurian, J. M. 5247:80; 5378:179

Gustafson, P. F. 5247:6, 24; 5288:129, 149; 5378:23, 26, 63

Hall, B. V. 5332:45

Halogen vapors
detection by alkali-sensitized positive ion emitters 5288:131

Hamilton, K. F. 5247:18; 5288:68; 5378:91, 100

Hammond, C. 5288:37, 83

Hamster
tissue mast cells 5378:123

Hartig, S. T. 5378:123

Hemolysin production in irradiated rabbits 5378:152

Hepatectomy
and ascites tumor growth 5378:71

High voltage supply 5378:62

Hill, M. S. 5378:71

Hill reaction 5247:44

Hilst, G. R. 5288:132, 150; 5378:63

Holloway, B. J. 5378:74

Human radium measurement 5378:27

Hydrogen peroxide
effect on Tetrahymena 5247:9
Hypophysis
and tissue mast cell in hamster 5378:123
and tissue mast cell in rat 5332:33

Hypotension
in chick response to radiation 5288:9

Immunity
effect of irradiation 5247:51

Indoleacetic acid (IAA) inactivation 5288:63

Indole-3-acetic acid oxidase 5332:61; 5378:128

Infection
endogenous, in irradiated mice 5288:37

Intracellular localization of
tryptophan-IAA system 5288:56

Inulin, C¹⁴-labeled
biosynthesis of 5332:100

Iodine
protein-bound 5247:24

Ion emission 5378:53

Ion pair
energy requirement 5378:30

Ionization
around point sources 5288:113
chambers for study of neutron
dose 5288:113
in C₄F₈ and air 5378:5
in pure gases 5378:30

Irradiation
and hemolysin production 5378:152
and phosphatase activity 5332:12
and renal function in rooster 5332:13
constant, effect on cell growth 5247:61
continuous, of tumors 5332:5
effect on immunity 5247:51
of biological materials and
carcinogenicity 5378:100
of bone with neutrons 5247:62
protection in mice 5332:79
protection by spleen 5378:173

Isotopes as tracers 5247:81

Jackson, E. M. 5247:7; 5288:40;
5378:167, 168
Jaroslow, B. N. 5247:51, 53;
5288:64; 5378:152, 182
Jesse, W. P. 5378:30, 63
Johnston, W. H. 5288:149
Jordan, D. L. 5247:30; 5288:35, 39;
5378:118
Katz, E. J. 5247:45
Katz, J. J. 5378:133
Kemp, N. E. 5332:52, 53, 78
Kidney shielding
modification of radiation syndrome
by 5247:8

Kinetics of glycine incorporation
(E.coli) 5378:159

Kisieleński, W. E. 5247:68; 5288:76

Koch, A. L. 5247:54; 5288:60, 61, 67;
5378:133, 140, 145, 148, 159, 162

Krebs tumor 5247:5

Kubitschek, H. E. 5288:65; 5332:94

Kulhanek, F. C. 5378:54

Laird, A. K. 5378:155

Lamont, W. A. 5288:60, 61, 67;
5378:133, 145

Leaves
light reflectance of 5247:10

Lesher, S. 5378:91

Lethality function
applied to toxicity of tritium
5288:16

Leucocytes
recovery after depletion 5247:7;
5378:168

regulation 5247:7; 5288:40

Leucopenia
and leucocyte anti-serum 5378:167

Lewis, Y. S. 5288:43; 5332:33;
5378:123

Light reflectance by plants 5247:10

Lindenbaum, A. 5247:65, 77;
5288:92; 5332:47, 90, 91

Lisco, H. 5247:25; 5288:23;
5378:106

Liver
acid phosphatase distribution 5247:46

function, tests 5247:45

regenerating, tryptophan
peroxidase and arginase 5378:150

Lucas, H. F., Jr. 5288:117, 149,
150; 5378:8, 16, 63

Lymphoma in hybrid mouse 5247:18

Maioney, M. A. 5247:7; 5288:40,
5378:167, 168

Malpighian tubules in grasshopper 5332:41

Mange
control in mice 5378:182

Marinelli, L. D. 5288:124, 129, 149,
150; 5378:5, 23, 27, 63

Mast cell
hamster, influence of hypophysis
and adrenal cortex 5378:123

number, of bat in different states 5288:43

rat, influence of hypophysis and
adrenal cortex 5332:33

May, H. A. 5288:113, 131, 150
Metabolic requirements for recovery of irradiated Tetrahymena 5247:9; 5288:84

Metabolism of chloroplasts 5247:44

Meteorology 5378:54

Meteorology stack 5288:132; 5378:54

Methylated purines mutagenic action 5288:67

5'-Methylthioadenosine preparation 5378:126

Microorganisms automatic counter 5332:94

Chilomonas paramecium 5247:43

Escherichia coli 5247:67; 5288:41; 5378:140, 159, 162

Paramecium 5247:55; 5332:75; 5378:152

Tetrahymena 5247:9, 43; 5288:84

Trypanosoma duttoni 5247:51

virus 5247:54

Migration of ions effect of sorption on 5288:69

Mikuta, E. T. 5247:46; 5288:62; 5332:12

Miller, C. E. 5288:124, 127, 149, 150; 5378:27, 62, 63

Miller, C. P. 5288:37, 83

Mitochondrial origin in Paramecium 5247:55

Mitochondrial structure in Paramecium 5332:75

Monitor alpha 5288:131

Monoergic neutrons production 5288:113

Morphology of radiation-damaged leaves 5288:53

Moses, H. 5288:132, 150; 5378:54, 63

Moss, E. M. 5378:79, 150

Moss, R. A. 5288:58; 5332:59

Mouse ascites formation in 5288:46

ascites tumor growth after hepatectomy 5378:71

cages, effect on mortality 5332:18

comparative biological effect of x-rays in 5332:7

daily probability of death 5378:118

effect of gamma-radiation 5247:30

effect of irradiation on immunity 5247:51

effect of neutron irradiation 5247:30
Mouse effect of streptomycin therapy 5288:83
endogenous infection 5288:37
fertility 5288:66
after x-ray 5378:156
hybrid, response to x-rays 5247:18
irradiated, effect of protective substances 5288:85
irradiation protection 5332:79
lethality genetic variation after irradiation 5288:68
mange control 5378:182
post-irradiation cataract formation 5332:29
radiation lethality and physical fitness 5378:83
Sr$^{89}$ and malignant bone tumors 5378:106
survival and aurintricarboxylic acid 5288:91
toxicity of Be 5247:76
toxicity of Sr$^{89}$ 5247:25
tumors in controls 5288:23
Moving effect on mouse mortality 5332:18
Mroz, E. A. 5378:50
Mutagenic action of methylated purines 5288:67
Mutants mean time of appearance in chemostat 5288:65
Neutron activation reactions 5247:80
dosimetry 5288:114, 116; 5378:7
effect on light reflectance in plants 5247:10
fast and cataract formation in mice 5332:29
and daily probability of death in mice 5378:118
thermal neutron contribution 5378:121
ionization chambers for study 5288:113
irradiation of bone 5247:113
lethality for mice 5247:30
monoergic, production 5288:113
Nitrogen, isotopic absorption and utilization of by buckwheat 5288:99; 5332:96
Normal population blood counts 5378:179
Norris, W. P. 5247:6, 62, 149;
5288:69, 79; 5332:66; 5378:25,
26, 74, 170
Nucleolus and nucleus relationship in Paramecium 5378:152

Nucleus role in protein synthesis 5378:155

Oxygen and radiosensitivity of grasshopper embryos 5288:88

Evolution in Hill reaction 5247:44

Paper electrochromatography 5288:69

Paramecium mitochondrial origin 5247:55

Mitochondrial structures 5332:75

Morphology 5247:55

Nucleolar and nuclear growth and form 5378:152

Radiation effects 5288:18

Particle accelerator 5288:113

Patt, H. M. 5247:5, 7; 5288:7, 40; 5378:83, 167, 168

Phantoms in x-ray dosimetry 5332:11; 5378:84

Phosphatase in rat liver 5247:46

In rat spleen and thymus after irradiation 5332:12

Phosphate compounds in bone 5247:62

Phosphate release temperature effect 5332:58; 5378:157

Phosphorus\(^{32}\) accumulation by Chlorella 5332:66 determination 5378:26

Phosphorus compounds effect of neutron irradiation 5247:62

Photochemical reduction role of thioctic acid and coenzyme A 5247:44

Photoperiodic stimulus in Xanthium 5332:39

Photoreactivation failure of dose-reduction principle 5288:41

Physical fitness and radiation lethality in mice 5378:83

Pitressin protection against radiation in chick 5247:72

Plant growth substitutions for boron 5332:54

Plasma proteins effect on irradiated ascites tumor cells 5378:176

Protection of irradiated mice by 5332:79

Plasma, mouse comparison with ascitic fluid in ultracentrifuge 5288:60
Plastics
in thin sections of bone 5378:170

Plumbism
effect of fluoroacetate 5378:178

Plutonium239
effect of zirconium on distribution 5332:82

Point sources
ionization around 5288:113

Poisoning
radioelement and nonradio-
element, treatment 5332:85

Poisons, metabolic
and radiosensitivity of grasshopper
embryos 5288:88

Polycyclic compounds
attempted synthesis 5288:95

Pomeroy, J. H. 5288:95

Positive ion emission 5378:53

Potassium
interference in radium measure-
ment 5378:27

Power for x-ray tubes 5378:62

Power supply
design 5288:131

Powers, E. L. 5247:55; 5288:8;
5332:75; 5378:152

Protective substances
effect on survival and weight
loss in irradiated mice 5288:85

Protein synthesis
role of nucleus 5378:155

Proton recoil spectrum
differentiation in the presence of
gamma-rays 5288:116

Pulse height spectrum of recoil
protons in presence of gamma-
rays 5288:114

Purines
isotope distribution (E.coli) 5378:140

Pyresis 5378:125

Rabbits
antibody formation 5247:53;
5288:64
hemolysin production after irra-
diation 5378:152

Rabideau, G. S. 5247:10, 44, 81;
5288:53; 5332:66

Radiation
and ascites tumors 5288:7
and hypotension 5288:9
and leaf damage 5288:53
and light reflectance of plant
leaves 5247:10
and F. aurelia 5288:8
and recovery from leucocyte
depletion 5247:7
and shielding in chick 5247:8
and tumor nuclei 5378:72
dosage
method for estimation in single-
chain, catenary system 5288:93
Radiation
lethality and physical fitness in mice 5378:83

protection by vasoconstrictor drugs 5247:72

Radioactinium
metabolism and distribution 5288:129

Radioautography of undecalcified bone 5378:170

Radiocarbon
growth and behavior of soybean induced by 5288:20

Radioruthenium
toxicity studies 5378:102

Radiothorium
apparatus for determining 5288:127
determination of traces in radium 5288:124

Radium
and Ca\textsuperscript{45} to dogs 5378:74

assay, gamma-ray 5288:129; 5378:27

content after inhalation of dust 5288:129

content of dogs 5288:129
determination of RaTh 5288:124, 127
dust, inhaled 5288:129

excretion 5288:117

Radium
intake and retention 5288:117;

metabolism in rats 5288:79

retention in man after I.V. dose 5247:6
toxicity 5288:129; 5378:23

Radon measurement 5288:117;

Rasmussen, P. 5288:64

Rat
influence of hypophysis and adrenal cortex on tissue mast cell 5332:33

liver particulates succinic dehydrogenase activity 5288:62

radium metabolism 5288:79

urea formation 5378:79

Recoil protons
determination of pulse height spectrum in presence of gamma-rays 5288:114

in neutron dosimetry 5288:116

Recoil spectrum
from neutron source 5378:7

Recovery from radiation metabolic requirements for Tetrahymena 5247:9

Relative biological effectiveness of fast neutrons and gamma-rays 5247:30
Renal function in irradiated rooster 5332:13

Ribonuclease treated with strong acids 5378:133

Rooster postirradiation renal function 5332:13

Rose, J. E. 5288:113, 129; 5378:27, 62, 63

Rosenthal, M. W. 5332:82, 92; 5378:125, 178

Roth, L. E. 5247:55; 5288:50; 5332:45, 75

Rowland, R. E. 5288:124, 149, 150; 5378:27, 62, 63

Rubber biosynthesis and turnover in Hevea 5288:100

Ruthenium toxicity 5247:23

Sacher, G. A. 5247:18; 5288:16, 41, 68; 5332:7; 5378:91, 179

Sadauskis, J. 5378:63

Sanderson, M. H. 5288:9, 66; 5332:13; 5378:76, 156

Sato, C. 5332:60

Sato, T. R. 5247:62; 5288:69; 5332:66

Schlenk, F. 5378:126

Schubert, J. 5247:77, 78, 79; 5288:92; 5332:82, 85

Schultz, H. A. 5288:131; 5378:53

Scintillation counter in double tracer experiment 5378:26

Scintillating solutions determination of recoil protons 5288:114

response 5378:7

Scorbutus and fibril formation in guinea pigs 5288:47

Scully, N. J. 5288:20, 100; 5332:100

Sectioning undecalcified bone 5378:170

Serine precursors 5247:67

Serum effect on irradiated ascites tumor cells 5378:176

heterologous, protection of irradiated mice 5332:79

Serotonin protection against radiation 5247:72

Shielding and weight of mouse spleen 5288:14

modification of radiation syndrome in chick 5247:8

Skok, J. 5288:99; 5332:54, 96

Smith, D. E. 5288:43; 5332:33; 5378:123
Soil
movement of water 5378:16

Sorption
effect on migration in paper electrophotography 5288:69

Soybean
radiocarbon effect on growth and development 5288:20

Specimen grid
for electron microscope 5332:45

Speckman, T. W. 5247:6; 5288:79, 129; 5378:25, 26, 74

Spleen
protection against irradiation 5378:173

protection of antibody-forming mechanism 5247:53

recovery after x-irradiation 5378:95

weight loss following irradiation 5288:14, 39

Stack
meteorology 5288:132; 5378:54

Stearner, S. P. 5247:8, 72; 5288:9, 38, 66; 5332:13; 5378:76, 156

Stehney, A. F. 5288:117, 149, 150; 5378:8, 16, 63

Story, V. M. 5247:30

Strain, H. H. 5288:69

Straube, R. L. 5288:7, 46; 5378:71, 83

Streptomycin therapy
effect on irradiated mice 5288:83

Strontium\textsuperscript{89}
malignant bone tumors in mice 5378:106
toxicity in mice 5247:25
toxicity study tumors among control animals 5288:23

Strontium\textsuperscript{89} - strontium\textsuperscript{90} mixtures, analysis 5288:76

Strontium\textsuperscript{90} - yttrium\textsuperscript{90} distribution and excretion in dog 5247:68
effect of point sources on tumor 5247:61

Stroud, A. N. 5247:61; 5288:14, 85; 5332:5, 79; 5378:72, 95, 176

Stutz, R. E. 5288:63; 5332:61; 5378:128

Succinic dehydrogenase activity of particulates after hepatectomy or CCl\textsubscript{4} 5288:62

Surgical trauma
and survival after irradiation 5378:76

Survival
effect of protective substances 5288:85

Svihla, G. 5288:43

Swanson, C. A. 5332:40
<table>
<thead>
<tr>
<th>Name</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swick, R. W.</td>
<td>5247:67; 5332:27; 5378:148</td>
</tr>
<tr>
<td>Tahara, D. M.</td>
<td>5247:67; 5332:27; 5378:148</td>
</tr>
<tr>
<td>Tahmisian, T. N.</td>
<td>5288:88; 5332:41; 5378:81, 154</td>
</tr>
<tr>
<td>Taliaferro, W. H.</td>
<td>5247:53; 5288:64, 5378:152</td>
</tr>
<tr>
<td>Taylor, L. S.</td>
<td>5288:149</td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
</tr>
<tr>
<td>effect on phosphate release</td>
<td>5332:58; 5378:157</td>
</tr>
<tr>
<td>measurement for interpretation of stack data</td>
<td>5288:135</td>
</tr>
<tr>
<td>Tetrahymena</td>
<td></td>
</tr>
<tr>
<td>effect of ethylmaleimide as antimitotic</td>
<td>5247:43</td>
</tr>
<tr>
<td>irradiated, metabolic requirements for recovery</td>
<td>5288:84</td>
</tr>
<tr>
<td>recovery from irradiation</td>
<td>5247:9</td>
</tr>
<tr>
<td>Thomson, J. F.</td>
<td>5247:46; 5288:50, 5332:12; 5378:79, 150</td>
</tr>
<tr>
<td>Thorium$^{237}$</td>
<td></td>
</tr>
<tr>
<td>metabolism and distribution</td>
<td>5288:129</td>
</tr>
<tr>
<td>Thymol turbidity and flocculation tests</td>
<td>5247:45</td>
</tr>
<tr>
<td>Tobacco</td>
<td></td>
</tr>
<tr>
<td>flavonoid studies in</td>
<td>5332:57</td>
</tr>
<tr>
<td>Toxicity</td>
<td></td>
</tr>
<tr>
<td>aurotricarboxylic acid-aluminum</td>
<td>5247:79</td>
</tr>
<tr>
<td>aurotricarboxylic acid-iron</td>
<td>5247:79</td>
</tr>
<tr>
<td>Be in mice</td>
<td>5247:76</td>
</tr>
<tr>
<td>ruthenium</td>
<td>5247:23; 5378:102</td>
</tr>
<tr>
<td>strontium$^{89}$</td>
<td>5247:25; 5378:106</td>
</tr>
<tr>
<td>Translocation</td>
<td></td>
</tr>
<tr>
<td>$^{14}C$-labeled compounds in grape</td>
<td>5332:40</td>
</tr>
<tr>
<td>Tritium</td>
<td></td>
</tr>
<tr>
<td>toxicity, lethality function</td>
<td>5288:16</td>
</tr>
<tr>
<td>measurement of water movement in soil</td>
<td>5378:16</td>
</tr>
<tr>
<td>Trypanosoma duttoni</td>
<td></td>
</tr>
<tr>
<td>radiation effect on immunity</td>
<td>5247:51</td>
</tr>
<tr>
<td>Tryptamine</td>
<td></td>
</tr>
<tr>
<td>as intermediate in auxin biosynthesis</td>
<td>5332:59</td>
</tr>
<tr>
<td>Tryptophan-indoleacetic acid system characterization</td>
<td>5332:60</td>
</tr>
<tr>
<td>cytoplasmic localization</td>
<td>5378:145</td>
</tr>
<tr>
<td>intracellular localization</td>
<td>5288:56</td>
</tr>
<tr>
<td>Tryptophan peroxidase in regenerating liver</td>
<td>5378:150</td>
</tr>
<tr>
<td>Tumors</td>
<td></td>
</tr>
<tr>
<td>activation analysis</td>
<td>5247:24</td>
</tr>
</tbody>
</table>
Tumors
bone, geographic studies 5332:6

continuous irradiation 5332:5
effect of Sr$^{90}$-Y$^{90}$ point sources on 5247:61
in control mice 5288:23
in mice after Sr$^{89}$ 5378:106
Krebs ascites, quantitative growth studies 5247:5
radiation effects on nuclei 5378:72

Tyler, S. A. 5288:93; 5378:14

Ultracentrifuge
comparison of mouse plasma and ascitic fluid 5288:60
patterns of treated proteins 5288:61

Urea formation
in irradiated, nephrectomized rats 5378:79
Vascular permeability
and ascites tumor 5378:71

Vasoconstrictor drugs
protection against radiation 5247:72

Velocity of electrons in gases 5378:40

Virus
absorption of by bacteria 5247:54

Vogel, H. H., Jr. 5247:30; 5288:35, 37, 38, 39, 83; 5332:29; 5378:118
Walton, H., Jr. 5247:23; 5332:7, 11; 5378:84, 100, 102, 121
Wassermann, F. 5288:47, 50; 5332:47, 48
Watanabe, R. 5288:97, 98; 5332:39
Water
movement in soil 5378:16

Weight loss
effect of protective substances 5288:85
Wender, S. 5332:57
Wendorf, G. 5288:41
Westfall, W. 5247:77; 5288:92; 5332:90, 91
White, M. R. 5247:76, 79; 5288:91
Wind vane 5288:134; 5378:58
Woodruff, L. A. 5378:170
Wright, B. J. 5378:81, 154

Xanthium
photoperiodic stimulus 5332:39

X-irradiation
and antibody formation in rabbits 5288:64
and cleavage time 5378:154
and genetic variation in lethality of mice 5288:68
X-irradiation and spleen recovery 5378:95
and weight and protein content of embryos 5378:81
low rate and surgical trauma 5378:76
protection of ascites tumor cells 5378:176

X-ray and mouse fertility 5288:66; 5378:156
and weight of mouse spleen 5288:14
comparative effect on BAF₁ mouse 5332:7
comparative effect on BAF₁ and CAF₁ mouse 5247:18

X-ray
effect on light reflectance in plants 5247:10
effect on yolk deposition in frog oocyte 5332:53
low energy, phantom dosimetry 5332:11
machine, modification of voltage control 5378:62
modification of effect in chick 5247:8
phantom dosimetry 5378:84
protection in chick 5247:72

Zerbe, G. A. 5378:54

Zirconium
effect on distribution of Pu²³⁹ 5332:82